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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ESTER DERIVATIVES OF 4-(DIETHYLAMINO) SALICYLALDEHYDE AS CHOLINESTERASE AND TYROSINASE INHIBITORS

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Abstract*:* *Alzheimer's disease, one of the diseases that still has no a specific therapy, has become a major public health issue owing to the increasing population of the elderly, particularly in rich countries. Inhibitory of cholinesterase enzymes (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which hydrolyze acetylcholine (ACh) and butyrylcholine (BCh) neurotransmitters, have recently become a choice for the therapy of this disease. Therefore, there is currently a great demand for novel enzyme inhibitors with desirable properties for applying in the treatment of AD. In this study, a series of ester derivatives of 4-(diethylamino)salicylaldehyde (**1-5)** were successfully prepared and structurally illuminated with FT-IR, ^{1}H- and ^{13}C NMR spectroscopy. The inhibitory properties of the synthesized molecules on AChE, BChE, and tyrosinase enzymes were investigated, respectively. Compound **1** indicated potent inhibitory activity against BChE with 87.28±0.87% inhibition better than galanthamine (73.83±0.25 %inhibition) employed as standard. Compound **3** showed significant inhibitory effect against tyrosinase with 87.73±0.22 % inhibition, which is better than kojic acid utilized as standard. The obtained results clearly revealed that some of these molecules have the potential to be used as potent enzyme inhibitor candidates in the future studies.*

Keywords*: Esters, Acetylcholinesterase, Butyrylcholinesterase, Tyrosinase.*

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1. Introduction

Alzheimer's disease (AD) is a progressive chronic disease that prevents the continuation of daily living activities [1]. The most important risk factor for AD is age. This disease affects the central nervous system in humans. AD causes cognitive and functional deterioration, neuropsychiatric and behavioral disturbances, visual and spatial skills, impairment in learning new information and remembering learned information, and speech impairment. In addition, AD is an irreversible neurodegenerative disease that causes significant impairment in a person's social and professional life [2,3]. AD is a disease that occurs with the loss of neurons and synapses in some parts of the central nervous system [4,5]. The earliest symptom of the disease is short-term memory loss. The first stages of the disease can be very mild, so this situation may not be noticed by the patient. Dementia is not a natural consequence of aging, but a disease that should be distinguished from the cognitive and

psychological changes seen with normal aging [6]. For its therapy, a treatment method is followed to prevent forgetfulness and related skill losses that reduce the quality of daily life. It is known that a decrease in ACh in the brain is one of the reasons why AD occurs in the elderly. Damage to the cholinergic system in AD is the most prominent neurochemical disorder known [7]. Among the pharmacological approaches developed to heal this damage, the most successful method is the treatment with AChE inhibitors. For the treatment process, AChE inhibitors are used to prevent the reduction of ACh that causes disease progression [8-12].

Melanin is one of the most common pigments in nature [13]. This pigment plays a role in the darkening of fruits and vegetables and also the determination of skin, hair, and eye color in humans and animals [14, 15]. Tyrosinase is the main enzyme responsible for the production of melanin pigment in fungi and vertebrates [16]. In living things, some pigmentation abnormalities can be observed in the skin due to the production of melanin and the activity of the tyrosinase enzyme [17,18]. Low activity of tyrosinase and decreases in melanin synthesis cause hypopigmentation problems in the skin [19]. However, excessive activity of the tyrosinase enzyme increases the amount of melanin synthesis, which leads to hyperpigmentation problems [20,21]. In both conditions, the differences in the parts of the skin that are visible and in contact with the social environment cause social and psychological problems in individuals. Nowadays, many people are interested in skin whitening (hypopigmentation agent) products [22]. In the dermo-cosmetics industry, especially in Asian countries, products with hypopigmentation effect are frequently preferred. The market for these products is growing as tyrosinase inhibitors not only remove pigmentation disorders but also other spots on the skin. Increasing interest from color cosmetics to functional cosmetics increases the investments made in this sector [23]. The molecules used as tyrosinase inhibitors can be obtained by chemical or biological pathways [24]. In some cases, an inhibitor obtained from biological sources is synthetically produced and used; for example, kojic acid [25].

This research was carried out to contribute to the ongoing researches for the discovery and development of effective enzyme inhibitors. In this study, we targeted to search the inhibition effects of the ester derivatives of 4-(diethylamino)salicylaldehyde (**1-5**) on cholinesterase and tyrosinase enzymes *in vitro* conditions. The synthesized molecules were characterized by spectral analysis such as FT-IR, ^{1}H, and ^{13}C NMR.

2. Materials and Methods

2.1. Chemistry and analysis

In the current study, all commercially available chemicals used for the synthesis of the target molecules were received from Turkish representatives of Aldrich, Sigma-Aldrich, and Merck Chemical companies. FTIR spectra of the synthesized molecules were recorded on Agilent Cary 630 FTIR spectrometer with diamond ATR imaging accessory. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz and 100 MHz (with $CDCl_3$ and DMSO-d_6 as solvent) spectrometer. Melting points of all ester derivatives were detected on a Barnstead IA9100 Electrothermal digital melting points apparatus.

2.2. General procedure for the synthesis of ester derivatives (1-5)

A solution of 4-(diethylamino) salicylaldehyde (10 mmol) and the corresponding benzoyl chloride derivative (10 mmol) in pyridine (25 mL) was heated under reflux for 1 h [26]. This solution was left to cool down and then poured onto ice-cold water (50 mL). Thereafter, it was held for 24 h at 25 °C, and the formed precipitate was filtered, and then rinsed with 50 mL of distilled water; and they

left to dry in the open air overnight. Finally, the residue was crystallized from ethanol to give the target molecule.

2.2.1 5-(Diethylamino)-2-formylphenyl benzoate (1) [27]

Shiny brown solid, yield: 88%, m.p. 90-92 °C. FT-IR, ATR (cm^{-1}): 3065 (C-$H_{arom.}$), 2971 (C-$H_{aliph.}$), 2817, 2712 (C-$H_{aldehyde}$), 1737 (C=O_{ester}), 1670 (C=$O_{aldehyde}$), 1598 (C=$C_{arom.}$). ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 6H, (2xC***H***$_3$)), 3.46 (q, 4H, (2xC***H***$_2$)), 6.43 (d, 1H, *J*=2.4 Hz, C_6H_3-***H***), 6.62 (dd, 1H, *J* = 8.9, 2.4 Hz, C_6H_3-***H***), 7.55 (t, 2H, *J*=7.7 Hz, C_6H_5-***H***), 7.65–7.70 (m, 1H, C_6H_5-***H***), 7.79 (d, 1H, *J*=8.9 Hz, C_6H_3-***H***), 8.24–8.28 (m, 2H, C_6H_5-***H***), 9.89 (s, 1H, –C***H***=O), ppm. ^{13}C NMR ($CDCl_3$) δ: 12.49 (***C***H_3), 44.85 (***C***H_2), 104.40, 108.79, 116.51, 128.66, 129.25, 130.33, 132.50, 133.75, 153.17, 154.62 (Ar-***C***), 165.09 (***C***=$O_{aldehyde}$), 186.23 (***C***=O_{ester}), ppm.

2.2.2 5-(Diethylamino)-2-formylphenyl 2-nitrobenzoate (2)

Pale yellow solid, yield: 85%, m.p. 86-88°C. FT-IR, ATR (cm^{-1}): 3095 (C-$H_{arom.}$), 2968 (C-$H_{aliph.}$), 2867, 2777 (C-$H_{aldehyde}$), 1747 (C=O_{ester}), 1652 (C=$O_{aldehyde}$), 1592 (C=$C_{arom.}$), 1526 (Ar-$NO_{2asym.}$), 1340 (Ar-$NO_{2sym.}$). ^{1}H NMR ($CDCl_3$) δ: 1.28 (t, 6H, (2xC***H***$_3$)), 3.49 (q, 4H, (2xC***H***$_2$)), 6.54 (d, 1H, *J*=2.4 Hz, C_6H_3-***H***), 6.63 (dd, 1H, *J*=8.9, 2.4 Hz, C_6H_3-***H***), 7.66–7.76 (m, 2H, C_6H_5-***H***), 7.81–7.87 (m, 1H, C_6H_3-***H***), 8.23–8.09 (m, 2H, C_6H_5-***H***), 9.79 (s, 1H, –C***H***=O), ppm. ^{13}C NMR ($CDCl_3$) δ:12.42 (***C***H_3), 44.93 (***C***H_2), 104.37, 108.78, 116.25, 124.10, 128.04, 130.28, 131.67, 134.89, 147.24, 152.66, 153.26 (Ar-***C***), 165.35 (***C***=$O_{aldehyde}$), 186.85 (***C***=O_{ester}), ppm.

2.2.3 5-(Diethylamino)-2-formylphenyl 3-nitrobenzoate (3)

Pale brown solid, yield: 89%, m.p. 106-107°C. FT-IR, ATR (cm^{-1}): 3087 (C-$H_{arom.}$), 2974 (C-$H_{aliph.}$), 2821, 2719 (C-$H_{aldehyde}$), 1752 (C=O_{ester}), 1672 (C=$O_{aldehyde}$), 1600 (C=$C_{arom.}$), 1525 (Ar-$NO_{2asym.}$), 1348 (Ar-$NO_{2sym.}$). ^{1}H NMR (DMSO-d_6) δ: 1.14 (t, 6H, (2xC***H***$_3$)), 3.45 (q, 4H, (2xC***H***$_2$)), 6.68 (d, 1H, *J*=2.4 Hz, C_6H_3-***H***), 6.75 (dd, 1H, *J*=8.9, 2.4 Hz, C_6H_3-***H***), 7.71 (d, 1H, *J*=8.9 Hz, C_6H_3-***H***), 7.92 (t, 1H, *J*=8.0 Hz, C_6H_5-***H***), 8.52–8.60 (m, 2H, C_6H_5-***H***), 8.78–8.80 (m, 1H, C_6H_5-***H***), 9.66 (s, 1H, –C***H***=O), ppm. ^{13}C NMR (DMSO-d_6) δ: 12.75 (***C***H_3), 44.65 (***C***H_2), 105.20, 109.13, 115.87, 122.67, 124.68, 128.66, 131.30, 135.21, 136.39, 148.39, 152.99, 153.33 (Ar-***C***), 163.27 (***C***=$O_{aldehyde}$), 187.15 (***C***=O_{ester}), ppm.

2.2.4 5-(Diethylamino)-2-formylphenyl 4-dinitrobenzoate (4)

Shiny dark yellow solid, yield: 92%, m.p. 131-132 °C. FT-IR, ATR (cm^{-1}): 3106 (C-$H_{arom.}$), 2975 (C-$H_{aliph.}$), 2821, 2717 (C-$H_{aldehyde}$), 1740 (C=O_{ester}), 1665 (C=$O_{aldehyde}$), 1600 (C=$C_{arom.}$), 1525 (Ar-$NO_{2asym.}$), 1344 (Ar-$NO_{2sym.}$). ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, 6H, (2xC***H***$_3$)), 3.47 (q, 4H, (2xC***H***$_2$)), 6.43 (d, 1H, *J*=2.4 Hz, C_6H_3-***H***), 6.65 (dd, 1H, *J*=8.9, 2.4 Hz, C_6H_3-***H***), 7.71 (d, 1H, *J*=8.9 Hz, C_6H_3-***H***), 8.37–8.44 (m, 4H, C_6H_5-***H***), 9.76 (s, 1H, –C***H***=O), ppm. ^{13}C NMR ($CDCl_3$) δ: 12.46 (***C***H_3), 44.91 (***C***H_2), 104.55, 108.72, 115.91, 123.71, 131.48, 135.02, 135.06, 150.84, 152.96, 153.11 (Ar-***C***), 163.36 (***C***=$O_{aldehyde}$), 186.57 (***C***=O_{ester}), ppm.

2.2.5 5-(Diethylamino)-2-formylphenyl 3,5-dinitrobenzoate (5)

Shiny dark yellow solid, yield: 96%, m.p. 149-150°C. FT-IR, ATR (cm^{-1}): 3058 (C-$H_{arom.}$), 2976 (C-$H_{aliph.}$), 2877, 2785 (C-$H_{aldehyde}$), 1749 (C=O_{ester}), 1675 (C=$O_{aldehyde}$), 1601 (C=$C_{arom.}$), 1540 (Ar-$NO_{2asym.}$), 1339 (Ar-$NO_{2sym.}$). ^{1}H NMR ($CDCl_3$) δ: 1.27 (t, 6H, (2xC***H***$_3$)), 3.49 (q, 4H, (2xC***H***$_2$)), 6.46 (d, 1H, *J*=2.4 Hz, C_6H_3-***H***), 6.67 (dd, 1H, *J*=8.8, 2.5 Hz, C_6H_3-***H***), 7.66 (d, 1H, *J*=8.8 Hz, C_6H_3-***H***), 9.32 (t, 1H, *J*=2.1 Hz, C_6H_5-***H***), 9.37 (d, 2H, *J*=2.1 Hz, C_6H_5-***H***), 9.66 (s, 1H, –C***H***=O), ppm. ^{13}C NMR ($CDCl_3$) δ (ppm): 12.42 (***C***H_3), 44.97 (***C***H_2), 104.71, 108.71, 115.41, 122.65, 130.15, 133.75, 136.81, 148.77, 153.13, 155.42 (Ar-***C***), 163.49 (***C***=$O_{aldehyde}$), 186.93 (***C***=O_{ester}), ppm.

2.3. Enzyme inhibition assays

Anticholinesterase assays

The inhibitory effects of synthesized molecules (**1-5)** on AChE and BChE were determined according to the modified spectrophotometric method of Ellman et al., [28]. In this study, the absorbance of each molecule was measured at 412 nm. The final solution of tested molecules was 200 µL. To calculate the percentage of both enzyme inhibitions, the following formula was employed:

$$\text{Inhibition (\%)} = (T_{control}-T_{sample}) / T_{control} \times 100$$

Where T is the absorbance. Galanthamine in this process was employed as a positive control. All tests were repeated three times.

Antityrosinase assay

Anti-tyrosinase activities of synthesized molecules (**1-5)** were determined according to the method designed by Hearing and Jimenez [29]. In this study, the absorbance of each molecule was measured at 475 nm. To calculate the percentage of all enzyme inhibitions, the following formula was employed:

$$\text{Inhibition (\%)} = (T_{control}-T_{sample}) / T_{control} \times 100$$

Where T is absorbance. Kojic acid was utilized as an inhibitor for the positive control. All tests were repeated three times.

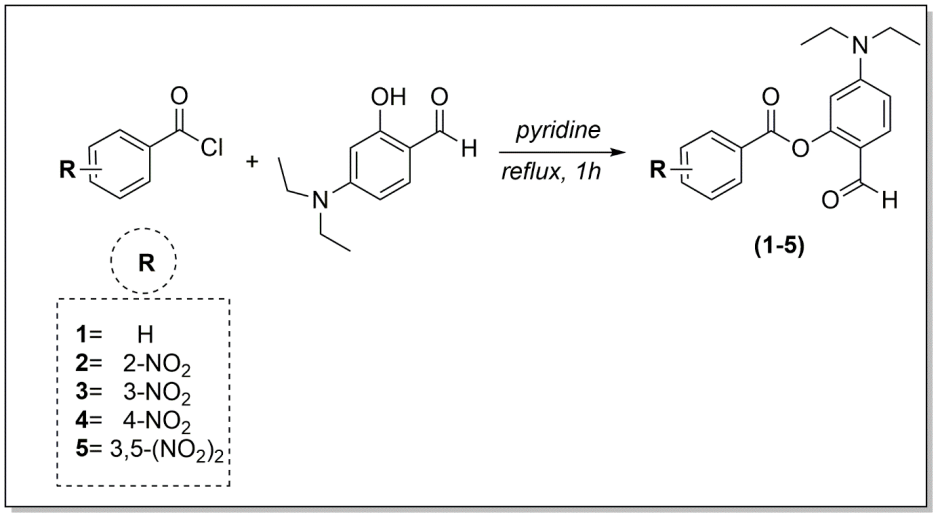
Statistical analysis

The results of antityrosinase and anticholinesterase activities of the synthesized molecules were reported as the mean ± standard deviation (SD) of three parallel measurements. The statistical significance was predicted by employing a Student's t-test, where a p-value < 0.05 was considered significant.

3. Results and Discussion

3.1. Synthesis and characterization

In this research, the ester derivatives of 4-(diethylamino)salicylaldehyde (one new, three commercially available, and one known) (**1-5)** as target molecules were successfully attained in one step. These esters were readily prepared by the reaction of 4-(diethylamino)salicylaldehyde with an appropriate non-substituted or nitro-substituted benzoyl chloride derivative in the molar ratio of 1:1 in pyridine as a solvent, which provided excellent yields (**85–96%**). To attain these molecules purely, they were thoroughly purified by crystallization method and their melting points were established. After these processes were performed, their chemical structures were illuminated with ^{1}H and ^{13}C NMR, FT-IR spectroscopic analysis, respectively. The synthetic pathway to obtain the target molecules (**1-5**) is described in Scheme 1.



Scheme 1. Synthetic pathway to synthesis of ester derivatives (**1-5**)

IR spectra of all synthesized ester derivatives showed strong absorption peaks in the 1737–1752 cm^{-1} range, representing the presence of the C = O group of the ester, while the C = O band of the aldehyde group was established at 1652–1675 cm^{-1}. A weak two-band signal of the aldehyde group (–CHO) was observed at 2817–2877 cm^{-1} and 2712–2785 cm^{-1}, respectively. Weak absorption bands detected in the ranges of 3058–3106 cm^{-1} and 2976–2968 cm^{-1} correspond to aromatic and aliphatic C–H stretching vibrations, respectively. Also, the symmetrical and asymmetrical stretching bands of the aromatic-nitro group (Ar-NO_2) in the structures of molecules (**2-5**) were observed at 1339–1348 cm^{-1} and 1525–1540 cm^{-1}, respectively. ^1H and ^{13}C NMR spectra of all synthesized target molecules were established in $CDCl_3$ (only compound **3** in DMSO-d_6) as solvent. In ^1H NMR spectra of all synthesized molecules (**1-5)**, the proton peak signal of the aldehyde group (HC = O) was observed as singlet peaks at 9.66–9.89 ppm. The proton peak signals of the aromatic rings were determined as a doublet, doublet of doublet triplet, or multiplet, between 6.43 and 9.37 ppm. Furthermore, the methyl protons (–CH_3) of the diethylamino group (–$N(CH_2CH_3)_2$) resonated as triplet peaks at 1.14–1.28 ppm, while methylene (–CH_2–) protons resonated as quartet peaks at 3.45-3.49 ppm. ^{13}C NMR spectra of all synthesized molecules (**1-5)** were determined to show different resonances in good agreement with the proposed structure. For the molecules (**1-5)**, C = O signals of the ester and aldehyde groups were detected at 186.23–187.15 and 163.27–165.35 ppm, respectively. In the spectra, the signals of the aromatic ring carbons in the aromatic region were observed between 104.37 and 154.62 ppm. Additionally, methyl (–CH_3) carbon atoms belonging to the diethylamino group were detected at 12.42–12.7549 ppm, and the methylene (–CH_2) signal was detected around 44.65–44.97 ppm. Moreover, FT-IR, ^1H, and ^{13}C NMR spectrum of the ester derivatives (**1-5**) were given in the supplementary material (Figures S1-S15).

3.2. Biological evaluation

In this study, the ester derivatives of 4-(diethylamino)salicylaldehyde (**1-5)** were made ready, and then their inhibition properties on AChE, BChE, and tyrosinase enzymes were screened. In inhibition studies, the synthesized molecules (**1-5)** on AChE showed inhibitory activities with IC_{50} values in the range of 24.43-58.46 μM. In AChE assay, galanthamine was employed to be the standard with a 79.40±0.46% inhibition value. Among the tested molecules, compound **4** indicated the best inhibition with a 58.46±0.81 % value in AChE inhibitory activity. Compounds **1**, **3**, and **4** showed moderate anti AChE activities (Table 1). In BChE assay, the tested molecules (**1-5)** on BChE demonstrated inhibitory activities with IC_{50} values in the range of 37.53-87.28 μM. Compound **1** displayed very strong activity against BChE enzyme with 87.28±0.87 inhibition% better than

galanthamine (73.83±0.25 % inhibition) employed as standard. Compounds **2** and **3** were also good anti BChE inhibitory activities (Table 1). On the other hand, these molecules (**1-5)** on tyrosinase enzyme displayed inhibitory activities with IC_{50} values in the range of 20.15-87.73 µM. Kojic acid was utilized as standard molecules in tyrosinase enzyme inhibitory activity with 79.76±0.79 % inhibition. Compound **3** showed quite strong tyrosinase enzyme inhibitory activity, with 87.73±0.22 % inhibition, which is better than kojic acid (Table 1).

Table 1. AChE, BChE and tyrosinase inhibitory results of the synthesized molecules

Inhibitors	AChE	BChE	Tyrosinase
1	46.55±0.65	87.28±0.87	53.08±0.54
2	34.88±0.73	62.22±0.75	26.64±0.50
3	47.50±0.31	70.74±0.22	87.73±0.22
4	58.46±0.81	37.53±0.54	47.51±0.97
5	24.43±0.40	49.01±0.97	20.15±0.30
Galantamine[b]	79.40±0.46	73.83±0.25	-
Kojik acid[b]	-	-	79.76±0.79

[a] 200µM
[b] Standard compounds

4. Conclusion

In this research, five the ester derivatives of 4-(diethylamino)salicylaldehyde were easily synthesized and characterized by by FT-IR, ^{1}H and ^{13}C NMR. In the biological activity studies, the inhibitory properties of all synthesized molecules were examined one by one. All tested molecules demonstrated enzyme inhibition activities against AChE, and the activity ordering of the synthesized molecules on AChE was determined as **4**>**3**>1>**2**>**5**. In AChE assay, compound **4** with an electron-withdrawing nitro group at the *para* position of the phenyl ring showed better activity than others. In BChE assay, the activity ordering of the synthesized molecules on BChE was determined as **1**>**3**>**2**>**5**>**4**. It was determined that compound **1** with a non-substituted phenyl ring had a very high activity even than standard compound. In tyrosinase assay, the activity ordering of the synthesized molecules on tyrosinase enzyme was established as **3**>**1**>**4**>**2**>**5**. It was found out that compound **3** with an electron-withdrawing nitro group at the *meta* position of the phenyl ring had a very high activity even than standard compound. When the structure-activity relationships of the tested molecules were investigated, we concluded that both non-substituted and nitro-substituted inhibitors generally had enzyme inhibition activities, although at different rates. Finally, we can easily say that compounds **1** and **3** are of great importance as potential agents for BChE and tyrosinase inhibitors, respectively.

Conflict of interest:

The article's authors declare that there is no conflict of interest between them.

The Declaration of Ethics Committee Approval

The author declares that this document does not require an ethics committee approval or any special permission. Our study does not cause any harm to the environment.

Authors' Contributions:

R. Ç: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft preparation, Writing - review&editing (%30)

E. Ç: Conceptualization, Validation, Investigation, Writing-original draft preparation (%25)

E. B: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft preparation, Writing - review&editing (%25)

M. B: Validation, Writing-original draft preparation (%20)

The compliance to Research and Publication Ethics

This work was carried out by obeying research and ethics rules.

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