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Araştırma Makalesi / Research Article

Investigation of Cholinesterase Inhibitor Effects of Some Chalcone Substituted Metallophthalocyanines

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Abstract

In this work, previously synthesized tetra-chalcone-substituted metallophthalocyanines (MPcs) containing Ni(II), Zn(II), Co(II), and Cu(II) in the inner core were investigated anticholinergic activities against Alzheimer's disease (AD). The MPcs were evaluated for their inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using the original Ellman's colorimetric procedure. Against these two cholinesterase enzymes, all compounds showed inhibitory effects with IC_{50} values ranging from 1.243-2.369 μM against AChE and 2.865-3.372 μM against BChE. Among the four MPcs, CuPcs (2) showed the strongest inhibitory activity against AChE. ZnPcs (3) also showed the most effective inhibition for BChE.

Keywords: Cholinesterases, Chalcone, Phthalocyanine, Inhıbıtor.

Bazı Kalkon Sübstitüe Metaloftalosiyaninlerin Kolinesteraz İnhibitör Etkilerinin İncelenmesi

Öz

Bu çalışmada, merkezde Ni(II), Zn(II), Co(II) ve Cu(II) içeren daha önce sentezlenmiş tetra-kalkon sübstitüe metaloftalosiyaninlerin (MPcs) Alzheimer (AD) hastalığına karşı antikolinerjik aktiviteleri incelenmiştir. MPcs'ler, orijinal Ellman'ın kolorimetrik prosedürü kullanılarak asetilkolinesteraz (AChE) ve butirilkolinesteraz (BChE) inhibisyonları açısından değerlendirildi. Bu iki kolinesteraz enzimine karşı tüm bileşikler, AChE'ye karşı 1.243-2.369 μM ve BChE'ye karşı 2.865-3.372 μM arasında değişen IC_{50} değerleri ile inhibitör etkiler göstermiştir. Dört MPcs arasında CuPc (2), AChE'ye karşı en güçlü inhibitör aktiviteyi göstermiştir. Ayrıca, ZnPcs (3), BChE için en etkili inhibisyonu göstermiştir.

Anahtar Kelimeler: Kolinesterazlar, Kalkon, Ftalosiyanin, İnhibitör.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that usually occurs in older age and results in impaired memory, language, and motor skills due to thinning of brain tissue. In this disease, behavior and thinking skills are impaired and gradually regress over the course of the disease phases. AD has three stages. Early, intermediate and advanced stages. Because symptoms are so mild in the early stages, the disease can be easily overlooked or confused with other conditions, such as depression. Although patients at this stage experience memory problems, they can perform routine tasks independently. Symptoms such as simple forgetfulness, the concept of time, difficulty remembering history, and regression in language skills are observed. In the middle stage, symptoms become more pronounced and the diagnosis is usually made at this stage. Cognitive impairments originating in the brain region responsible for speech and language become more frequent; patients have difficulty repeating learned meaningful movements. In the advanced stage, the third stage, patients develop urinary incontinence, motor disorders, postural impairments, and difficulty walking. During this period, patients become almost completely dependent on nursing care (Breijyeh and Karaman, 2020). Despite numerous scientific efforts, a definitive treatment method that eliminates Alzheimer's or halts its progression has not yet been found (Erdoğan et al., 2021). In addition, there are some drug and non-drug methods to slow the onset and progression of the disease.

As the cause of AD is still unknown, there is no precise cure for its. However, common factors such as such as cholinergic hypothesis agglomeration of β-amyloid (Aβ) peptides, τ-protein aggregation and oxidative stress have been found to trigger the disease. Inhibition of AChE and BuChE enzymes that hydrolyze acetylcholine (ACh) and butyrylcholine (BCh) neurotransmitters have become a treatment option for AD (Lima et al., 2020; Shaikha et al., 2020; Kazancıoğlu et al., 2020). AD is directly related to the decrease in the level of the cationic neurotransmitter Acetylcholine (ACh). During the progression of AD, many dissimilar types of neurons decay, although there is a significant loss of fore brain cholinergic neurons, which is attented by a decrease in acetylcholine. One of the most studied proteins as a target for AD therapy is the cholinesterases (Panek et al., 2018).

The chalcone fragments are widely available in both natural and synthetic product. They are significant intermediates in organic synthesis. (Tosun et al., 2015). Studies have shown that chalcones possess important pharmacological characteristics including antitumor, anti-inflammatory, antifungal and antioxidant properties. (Fu et al., 2016). Both metall-free and metallophthalocyanines (Pcs), which have very good thermal and chemical stability are important class of tetrapyrrole compounds. Due to they have a much conjugated π-electron system, commonly

have been used in dissimilar areas of technology and medicine. (Rey et al., 2018; Medina, et al., 2019).

Both chalcones and phthalocyanine analogs have become the focus of interest in recent years with their medicinal applications. They show widespread biological activity such as antibacterial, α-glucosidase, antioxidant, carbonic anhydrase (CA I, II, IX), inhibition of cholinesterase (AChE / BChE) (Çakır and Arslan, 2022; Barut et al., 2020). They have also become attractive in medicine to develop compounds with high potential to cure diseases. In our recent studies, we reported that compounds with a similar structure are also active against some esterase enzymes (for both hCAs and AChE). (Arslan 2021; Arslan et al., 2020). In literature, Keleş et al. synthesized water soluble phthalocyanines containing ({6-[3-(diethylamino)phenoxy]hexyl}oxy groups and tested their inhibitory effects on cholinesterases. They found that these phthalocyanines indicated significantly inhibitory actions effects than galantamine which was used as a positive control (Keleş et al., 2022). Recently, Öztürmen et al., reported in vitro α-glucosidase, cholinesterases (AChE / BChE) and tyrosinase inhibitory effects of 3,3-diphenyl-propoxy substituted silicon and Cu / Mn phthalocyanines.(Öztürmen et al., 2022)

Considering the literature information and as a continuation of our research for new anti-Alzheimer targets, we aimed to investigate already synthesized phthalocyanines with chalcone moiety. We first resynthesized the chalcone compound, the phthalonitrile compound and its peripherally tetra-substituted metallophthalocyanine (2-5) in light of the literature (Çakır, 2020). The inhibitory effects of (2-5) were investigated for the first time using The original Ellman's colorimetric procedure against cholinesterase enzymes (AChE and BChE) to determine the therapeutic potential.

2. Materials and Methods

2.1. Chemical Synthesis

Tetra-chalcone-substituted metallophthalocyanines were resynthesized according to the previous study in the literature (Çakır, 2020). All spectral data of the newly synthesized phthalocyanines are consistent with the literature. All chemicals were purchased from commercial suppliers and used without further purification.

2.2. Biochemical studies

The inhibitory effect of the synthesized compounds on acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes was determined by the modified spectrophotometric method of Ellman (Ellman et al., 1961). Rivastigmine was used as a reference substance for the assay. First, the compounds were dissolved in DMSO at a concentration of 1 mg/ml and then diluted to different concentrations with deionized water. Six serial dilutions of the inhibitors were measured to determine the cholinesterase inhibitory activity. For the cholinesterase inhibition experiments, 60 µL of buffer (0.1 M, pH 8.0; Tris-HCl buffer for the AChE assay and 0.1 M, pH 7.8; phosphate buffer for the BChE assay), 30 µL 2 mM DTNB, 1 µL enzyme (0.28 units/mL for the AChE assay and 0.32 units/mL for the BChE assay) and 5-30 µL inhibitor sample were added to a 96-well microplate. The reaction mixtures were incubated for 10 minutes at room temperature. Then, 30 µL of 2 mM (AChI/BChI) was added to initiate the enzyme reaction, and the reaction mixtures were incubated at room temperature for 10 minutes. Absorbance was measured three times within 5 min at 412 nm using a 96-well microplate reader (AccuReader M965, Metertech) and results are reported as mean ± standard deviation. IC_{50} values were determined using GraphPad Prism 5 software by running an inhibitor curve against the normalization of the response (variable slope). IC_{50} is the concentration at which a substance exerts half of its maximum inhibitory effect (Almaz et al., 2021).

3. Findings and Discussion

Previous studies by our group investigated the inhibitory effects of metal-free and metallo-phthalocyanines with different substituents against cholinesterases (Arslan et al., 2020). In these studies on molecules, it was found that they showed very good inhibitory effects on cholinergic enzymes. Therefore, it was decided to study the phthalocyanines (2-5) to determine new inhibitors against these enzymes in molecules with similar structures. We also report here the first study of the inhibitory effect of these phthalocyanines (Figure 1) on cholinergic enzymes (AChE and BChE). The IC_{50} values of the molecules are listed in Table 1.

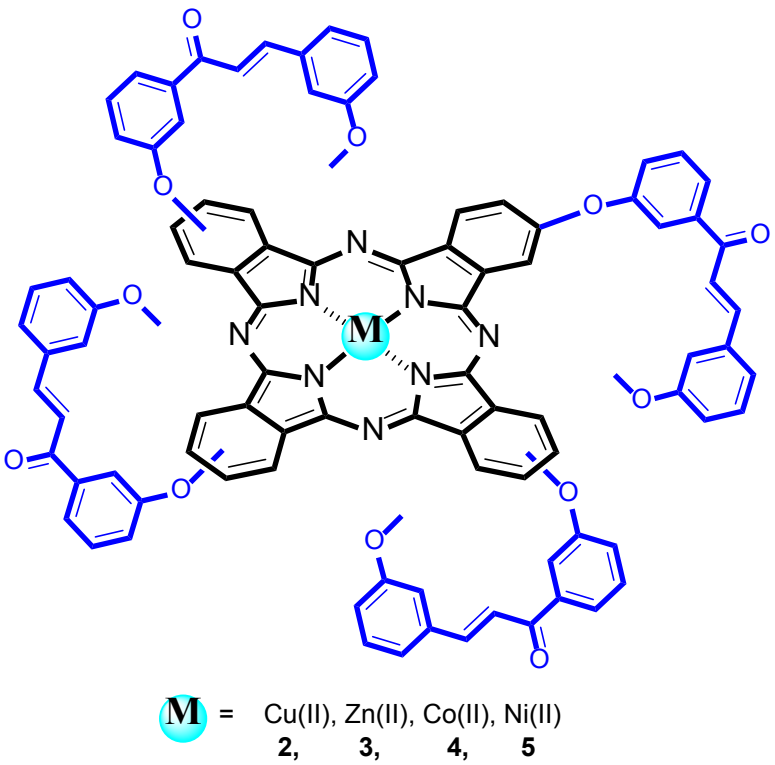

Figure 1.The tetra-chalcone substituted Cu(II), Zn(II), Co(II), Ni(II) phthalocyanines.

According to the cholinergic hypothesis in the etiology of AD, it has been found that increasing acetylcholine levels in patients relatively slows down symptoms. Scientific studies have shown that cholinesterase inhibitors have been successfully used in the treatment of neurodegenerative diseases such as AD. Among them, the most commonly used drugs are cholinesterase inhibitors such as rivastigmine, donepezil, and galantamine (Srivastava et al., 2021). In addition, there are studies in the literature using substituted phthalocyanine and chalcone analogs as inhibitors against AD. In a work by Arslan et al. the inhibitory activities of tetra-chalcone substituted metal-free and metallo-phthalocyanines on the enzyme AChE were investigated, which are associated with AD. In a recent work, three new silicon phthalocyanine derivatives were shown to be potent AChE and BChE inhibitors (Çakır and Arslan, 2022).

Table 1. IC_{50} values for AChE and BChE (μM) *

Inhibitor	AChE	BChE
2	1.243 ± 0.11	2.936 ± 0.07
3	1.466 ± 0.09	2.865 ± 0.08
4	1.592 ± 0.08	3.372 ± 0.13
5	2.369 ± 0.15	3.307 ± 0.12
Rivastigmine **	3.01	0.3

*Mean from at least three determinations.
**Rivastigmine were used as a control for AChE and BChE (Ozten et al., 2021).

(i) The IC_{50} values of the inhibitors ranged from 1.243 to 2.369 μM in inhibition studies of the AChE enzyme. The strongest inhibition was observed with tetra-chalcone substituted copper(II)-phthalocyanine (2) (IC_{50} = 1.243 μM) compared to the others. It was found that NiPc (5) showed the least inhibitory effect with an IC_{50} value of 2.369 μM. According to these results, all Pcs showed good IC_{50} values compared with rivastigmine (IC_{50} = 3.01 μM). The IC_{50} values of their continued the order 2 > 3 > 4 > 5 for AChE.

(ii) In the studies performed on BChE, IC_{50} values ranged from 2.865 to 3.372 μM. Among these phthalocyanines, Cu and Zn phthalocyanines (IC_{50} = 2.936 μM and 2.865 μM, respectively) showed moderate inhibition compared with the reference drug rivastigmine (IC_{50} BChE = 0.3 μM), a commercially available cholinesterase inhibitor. Ni and Co phthalocyanines (IC_{50} = 3.307 μM for NiPcs and 3.372 μM for CoPcs) also showed a low effect.

Considering the data obtained from our previous studies, the change of metals in the center of phthalocyanines has a great influence on their inhibitory ability. All phthalocyanines (2-5) showed different inhibitory effects on cholinesterase enzymes. When the IC_{50} values for AChE and BChE were examined, 4 and 5, which contained nickel and cobalt in the core, showed the least inhibitory effect. An improvement in the inhibition effect of 2 and 3 containing copper(II) and zinc(II) in their core, respectively, was also observed. As described in the literature, our results in this study also showed that the presence of different metals in the core of the phthalocyanine molecule can affect their inhibitory activity. This could be due to different interactions with enzymes as a result of the change in electron density of phthalocyanines (Yalazan et al., 2020; Tian et al., 2009; Arslan et al., 2020).

The active site of AChE consists of two binding subunits. The first is CAS, the catalytic active site, and the other is PAS (peripheral anionic site) (Kazancıoglu and Sentürk, 2020; Rosenberry et al., 2017). The mechanisms theoretically proposed in previous studies are shown in Figure 2. It can be easily predicted that the oxygen atoms of the carbonyl and methoxy groups in the chalcone structure interact with the AChE active sites, as is the case with classical cholinesterase inhibitors.

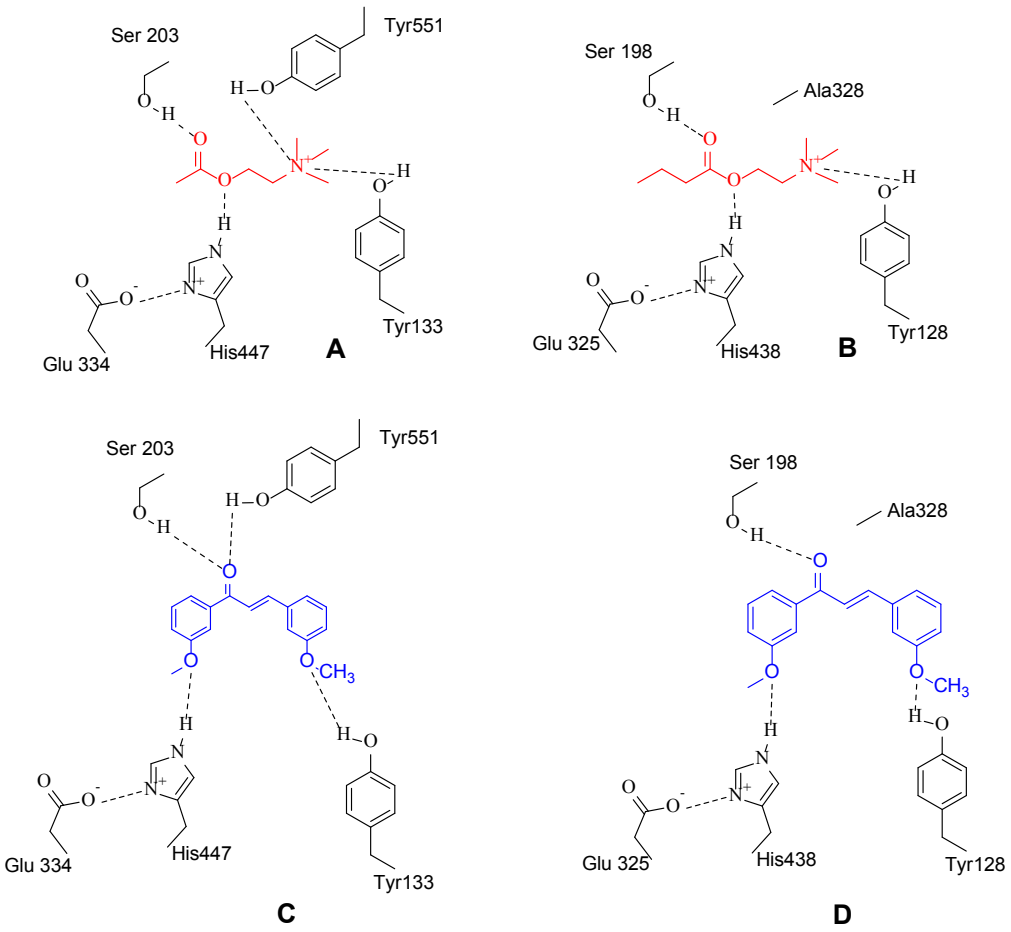

Figure 2. A and B: The binding interactions of acetylcholine and butyrylcholine with CAS (for the hAChE and hBChE), C and D: The estimated binding model of CuPcs (2) for both AChE and BChE, respectively.

4. Conclusions and Recommendations

In summary, inhibition of AChE and BChE by phthalocyanines peripherally substituted with tetrachalcone (2-5) was reported for the first time. The copper (II) (2) and zinc (II) (3) phthalocyanines were each good inhibitors of cholinesterase enzymes. These results indicated that methoxylated chalcone phthalocyanines could form the basis for the development of new anticholinesterase inhibitors.

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Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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