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Screening of Inhibitory Effects Lanosterol on Butyrylcholinesterase, Acetylcholinesterase and Carbonic Anhydrase Enzymes

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Highlights:

- High inhibition
- Enzymes
- Lanosterol

Keywords:

- Lanosterol
- Inhibition
- CA I-II isoenzymes
- Acetylcholinesterase
- Butyrylcholinesterase

ABSTRACT:

In this study, for the first time, the antiglaucoma and anticholinergic properties from lanosterol were appraised and researched using different bioanalytical methods and compared with standards. Lanosterol is the compound from which entire fungal and animal steroids are derived. Moreover, is a tetracyclic triterpenoid. Lanosterol is a component in over-the-counter ophthalmic products to prohibit cataracts. The inhibition effects of lanosterol were tested against the butyrylcholinesterase (BChE), carbonic anhydrase I and II (CA I and II) and acetylcholinesterase (AChE), which are associated with some global diseases like Alzheimer's disease (AD) and glaucoma. Lanosterol were trialed for the inhibition of BChE, AChE, hCA I and II enzymes and indicated efficient inhibition profiles with Ki values in the range of 61.77±22.32 nM against hCA I, 101.11±49.74 nM against hCA II, 2.03±1.21 nM against acetylcholinesterase and 8.39±2.92 nM against butyrylcholinesterase.

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INTRODUCTION

Carbonic anhydrases (CAs, E.C. 4.2.1.1) are enzymes in the family of metalloenzymes that catalyze the reversible hydration of carbon dioxide (CO_2) and water (H_2O) to a proton (H^+) and bicarbonate (HCO_3^-). (Ahmed, 2020) (Figure 1). This reaction is concerned to very important physiological mechanism, including the deregulation of carbonic anhydrases enzyme activity has been connected to diseases like cancer, obesity, glaucoma and epilepsy (Ahmed, 2019; Saeed, 2014). Carbonic anhydrases have recorded as ultimate medicament destinations for the design of specific activators or inhibitors with medical application. Carbonic anhydrase enzymes inhibition has been specified as the expectant initial step in the cure of a range of disorders like cancer, obesity, epilepsy and glaucoma (Imran, 2015; Turkan, 2019).

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

At every phase of life, we need enzymes. At the molecular level, thousands of reactions take place through enzymes. Acetylcholinesterase (E.C.3.1.1.7) is one of these enzymes (Göçer, 2013; Akıncıoğlu, 2014). As seen in Figure 1, cholinergic receptors (muscarinic receptors (MR), acetylcholine (ACh), butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), nicotinic receptors (NR)) and cholinecetyltransferase (ChAT) are components of the cholinergic system. The first neurotransmitter identified was acetylcholine (Tripathi, 2008). Acetylcholine (ACh) is a chemical transmitter of the central nervous system. It is also involved in the parasympathetic nervous system of many organisms (Göçer, 2016). When ACh is released in the synaptic space, AChE quickly terminates the resulting neural impulse. AChE removes ACh from the synaptic gap by catalyzing the hydrolysis of acetylcholine to choline and acetate (Schumacher, 1986). This enzyme is a five hundred thirty seven amino acid long peptide monomer talented of hydrolyzing 250,000 ACh molecules in one second (Sussman, 1988). Butyrylcholinesterase is the enzyme that catalyzes the hydrolysis of butyrylcholine, butyrate and choline in metabolism.

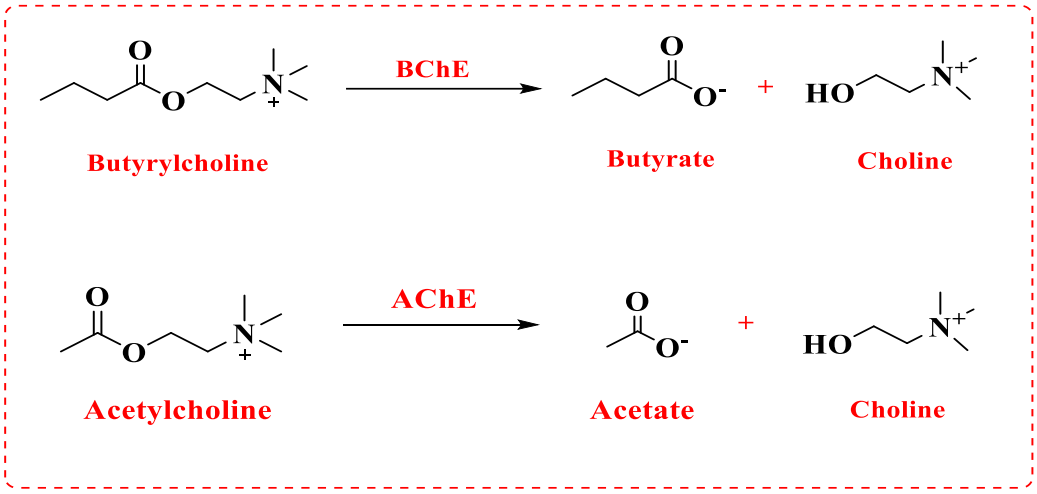


Figure 1. Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) hydrolysis mechanism

Alzheimer's disease (AD) was defined in 1906 by Dr. Alois Alzheimer (Arsava, 2003). It is a progressive neurodegenerative disease that occurs due to loss of synapses and neurons in various parts of the central nervous system and is characterized by self-care deficiencies, decreased cognitive functions, and various neuropsychiatric and behavioral disorders (Lieo, 2006). Acetylcholinesterase inhibitors (AChEIs), anticholinesterases are used as the most effective drug group in the treatment of AD. Anticholinesterase drugs inhibit the hydrolysis of acetylcholine, an important neuromodulator of the central nervous system, by reversibly or irreversibly inhibiting AChE (Jeger, 2013). Furthermore,

butyrylcholinesterase can hydrolyze acetylcholine. BChE levels do not decrease in AD disease, there is a possibility that this enzyme may increase (Taslimi, 2017; Köse 2015). In the brains of healthy individuals acetylcholinesterase is dominant however butyrylcholinesterase activity rise while acetylcholinesterase activity remains unmodified or reduce in the brain of Alzheimer's disease (Topal, 2017; Koca, 2023). BChE and AChE are liable for the termination of cholinergic signaling by hydrolyzing acetylcholine. Thus, a medicament inhibiting this enzyme might be preferable to selecting cholinesterase inhibitors. Nowadays, as give in Figure 2, synthetic cholinesterase inhibitors, including donepezil, rivastigmine, galantamine and tacrine have been used for clinical cure of AD When the brain acetylcholine levels of healthy individuals and Alzheimer's patients were compared, the difference was calculated to be around 50% (Gulcin, 2016).

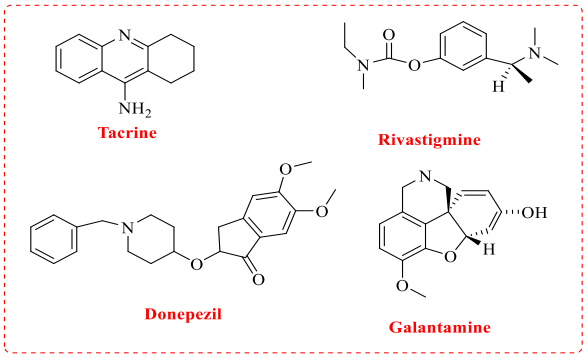


Figure 2. Commonly used cholinesterase inhibitors

Lanosterol is the compound from which all fungal and animal steroids are reproduced (Figure 3). Moreover, is a tetracyclic triterpenoid (Schaller, 2003). The structure of Lanosterol (Lanosta-8,24-diene-3β-ol) was elucidated by classical methods in 1950 by a Swiss team led by W. Voser (Voser, 1950). Lanosterol plays an important physiological role such as potential activity in cataract treatment (Kolesnikova, 2006; Chen, 2018; Yang, 2018), a metabolic weakness of resistant cells (Staubert, 2016; Nes, 2000) and cancer prevention activity (Rao, 2002). The human eye lens consists of crystal proteins assembled into a highly layout, interacting macrostructure essential for refractive index and lens transparency (Pascolini, 2010; Zhao, 2015). Protein aggregation is the major factor causing cataract. Mutations in oxidative stress are among the factors that cause protein aggregation. (Moreau, 2012). More than half of the blindness cases in the world are caused by cataracts. The treatment of cataract, curtain descending to the eye, is accomplished by surgically removing the opaque lens. In developed countries, cataract surgeries constitute a significant portion of healthcare costs as this disease is quite common among aging populations (Bloemendal, 2004). In Pre-clinical studies have shown which can reverse the aggregation of intracrystalline proteins that lanosterol can be used qua a feasible agent for the prevention of cataracts and is a potential drug for cataracts treatment (Zhao, 2015). In 2018, lanosterol was reported to improve lens clarity in cells with lens clouding due to aging or physical stressors (Shen, 2018). Another study positive results were also obtained as a result of studies on mice with cataracts in the lens of the eye (Wang, 2022). Experiments on dogs indicated important reversal of cataracts of six weeks of lanosterol injection (Zhao, 2015). Another in vitro result revealed that lanosterol can also reduce the aggregation of lens proteins, which greatly improves the clarity of the rabbit opaque lens (Zhao, 2015).

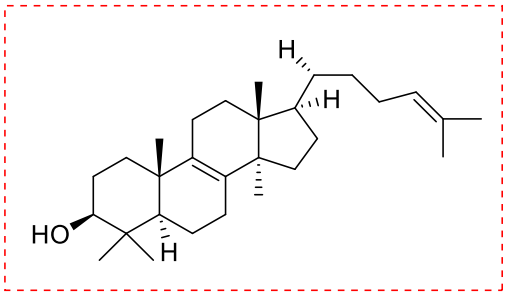


Figure 3. The chemical structure of lanosterol

MATERIALS AND METHODS

Antiglaucoma (hCA I-II) Inhibition Assay

Human erythrocytes were centrifuged at 10000x g for half an hour at 25°C to separate the serum fraction from other blood components. The pH of the serum fraction was set to 8.7 using solid (hydroxymethyl)aminomethane (Burmaoglu, 2022). Isoenzymes were purified to high purity using the Sepharose-4B-L-Tyrosine-sulfanilamide affinity column method (Kucuk, 2016; Akıncıoglu, 2014). The purity of hCA I-II isoenzymes was defined using sodium dodecyl sulfate–PAGE method, as explained in our previous work (Mutlu, 2023). Isoenzyme's inhibition and purification studies, according to the esterase activity method, the absorbance values were measured spectrophotometrically at 348 nm. (Kocyigit, 2018; Mirzazadeh, 2021).

Anticholinergic (BChE-AChE) Assay

The inhibitory effect of lanosterol against butyrylcholinesterase and acetylcholinesterase enzymes was performed according to the Ellman's process (1936) with certain modifications (Koca, 2023). Absorbance measurements values were recorded at 412 nm and DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)), BChI (Butyrylcholine iodide), AChI (Acetylthiocholine iodide) were used as substrates for the enzymatic reaction (Bal, 2021; Behcet, 2018).

Determination of IC_{50} Values

The IC_{50} values were calculated from activity (%) against lanosterol (Durmaz, 2022). Lineweaver-Burk plots were used to determine the Ki values (Bora, 2022).

RESULTS AND DISCUSSION

In this study we carried out focuses on biological activity, a widespread problem in medicine and other related fields. Physiologically hCA I, hCA II, BChE and AChE were researched in the enzyme inhibition. In drug studies for the treatment of antiglaucoma disease hCA II isoenzyme, for retinal and cerebral edema CA I enzyme was selected and studies were carried out (Ceylan, 2017; Göcer, 2017). For the CA I- II isoenzymes, lanosterol had Ki values of 61.77±22.32 and 101.11±49.74 nM, and had IC_{50} values of 49.50 and 69.30 nM, in order of. Acetazolamide, used as a CA inhibitor for the medical therapy of dural ectasia and idiopathic intracranial hypertension, altitude sickness, glaucoma, for CA I and II, showed Ki values of 26.29±6.83 and 37.70±9.79 nM, respectively. Butyrylcholinesterase and acetylcholinesterase were strongly inhibited by lanosterol compound. For the AChE and BChE, lanosterol had Ki values of 2.03±1.21 and 1.35±0.54 nM, and had IC_{50} values of 2.24 and 2.68 nM, in order of. Studies were carried out by selecting Tacrine as the positive control inhibitor for BChE and AChE enzymes; it had Ki values of 54.84±15.83 and 8.39±2.92 nM,

respectively (Table 1). It was concluded that Lanosterol was as effective as standard acetazolamide in inhibiting hCA I-I, and was more effective than the standard Tacrine in inhibiting AChE and BChE.

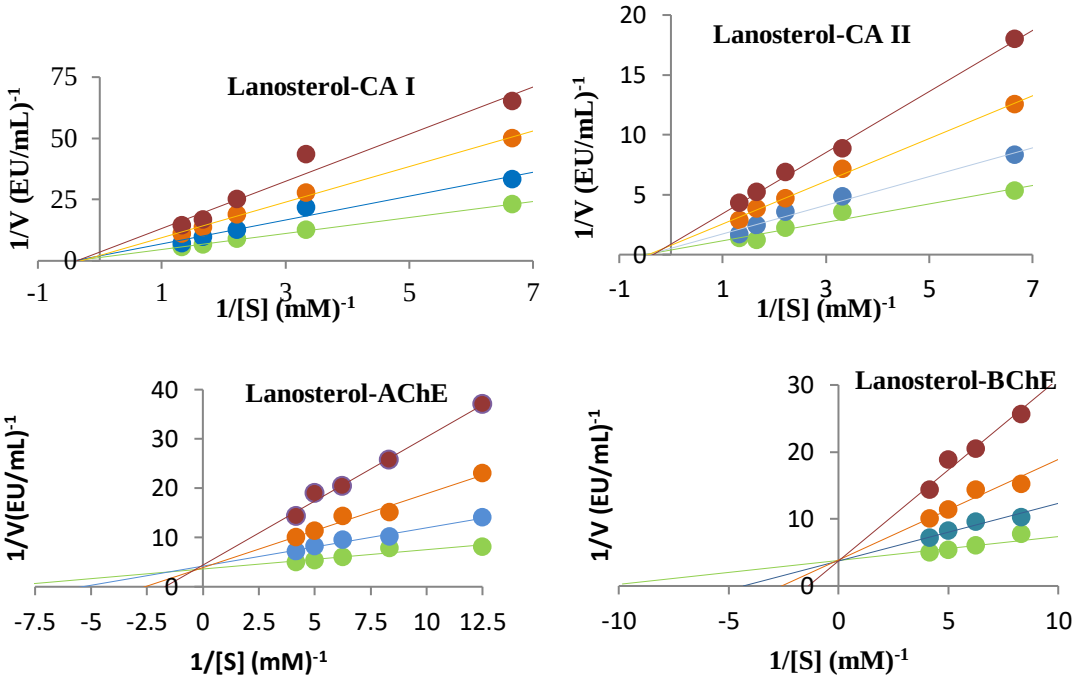
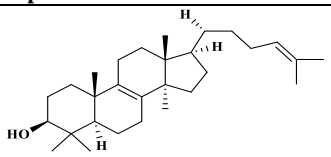
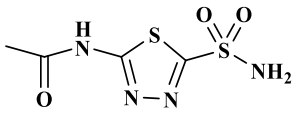
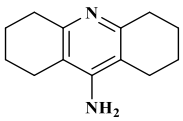


Figure 4. Inhibitory effect of Lanosterol on BChE, AChE and CA I-II isoenzymes activity

Table1. The enzyme inhibition results of lanosterol against metabolic enzymes

Compounds		CA I	CA II	AChE	BChE
 Lanosterol	IC_{50}(nM)	49.50	69.30	2.24	2.68
	K_i (nM)	61.77±22.32	101.11±49.74	2.03±1.21	1.35±0.54
	r^2	0.9937	0.9658	0.9047	0.9618
 Acetazolamide	IC_{50}(nM)	43.32	53.31	-	-
	K_i (nM)	26.29±6.83	37.70±9.79	-	-
	r^2	0.9921	0,9502	-	-
 Tacrine	IC_{50}(nM)	-	-	13.58	22.35
	K_i (nM)	-	-	54.84±15.83	8,39±2,92
	r^2	-	-	0.9401	0.9889

The role of hCA in diseases like epilepsy, edema, obesity and glaucoma has thoroughly been defined. Hyper-secretion of watery humor in the eye reasons enhanced intra-ocular pressures. Finally leading to a situation named as glaucoma (Göçer, 2016; Beydemir, 2004; Akıncıoğlu, 2015). Decline in carbonic anhydrases activity reduces the aqueous humor and secretion of HCO_3^-, thereby decreasing the pressure (Kose, 2016; Taslimi, 2016). AChEIs are the most efficient approach to therapy the cognitive signs of AD and other important therapeutic applications in the treatment of Parkinson's disease, ataxia and senile dementia (Kocyigit, 2017). Lanosterol inhibited the metabolic enzymes at

nanomolar levels. Therefore, inhibition of hCA isoforms and AChE can have a significant role in drug design and discovery as well as in medicine and toxicology. Also, lanosterol is a drug candidate as an antiepileptic and anticholinergic.

CONCLUSION

Lanosterol can be choosing inhibitor of butyrylcholinesterase, acetylcholinesterase and carbonic anhydrases I and II isoenzymes. Lanosterol can be admissible nominee drugs, the same as carbonic anhydrases inhibitors, for therapy of diseases like osteoporosis, glaucoma, epilepsy and neurological disorders. AD is the common type of dementia in the elderly population, is among the top 10 causes of death worldwide (Li, 2017). AD treatment can be performed by increasing the acetylcholine level in the brain by inhibiting the AChE present in the brain. BChE and AChE play critical roles in the hypothesis of cholinergic harm, and taking BChE and AChE as targets is absolutely the important to the studies on new Alzheimer's disease drugs.

Conflict of Interest

The author declared that there is no conflict of interest.

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