SHORT REPORT

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Inhibition Effects of Some Lignans on Carbonic Anhydrase, Acetylcholinesterase and Butyrylcholinesterase Enzymes

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Abstract: Lignans are a large group of chemical compounds found in plants. They have effects on enzymes, protein synthesis, cell proliferation, angiogenesis, growth factor and cell differentiation. In this study, inhibition effects of α-(-)-conidendrin, enterodiole, enterolactone, nordihydroguaiaretic acid, secoisolariciresinol and secoisolariciresinol diglucoside against carbonic anhydrase I and II isoenzymes (CA I, and II) and acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were investigated. The Ki values of the lignans were found to be in the ranges of 1.27-3.30 nM for CA I, 1.11-2.68 nM for CA II, 0.72-1.62 nM for AChE, and 0.08-0.20 nM for AChE.

Keywords: Lignans; acetylcholinesterase; butyrylcholinesterase; carbonic anhydrase.

1. Sample Source

Lignans, possessing a 2,3-dibenzylbutane skeleton, are present in glycoside form in many plants and widely found in flaxseed, rye, cherry, berries, grain, whole wheat, vegetables, fruits and tea [1]. α-(-)-conidendrin (**1**), enterodiole (**2**), enterolactone (**3**), nordihydroguaiaretic acid (**4**), secoisolariciresinol (**5**), and secoisolariciresinol diglucoside (**6**) are used in order to determine the inhibition effects on carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase enzymes (Supporting information S1, Figure S1).

2. Previous Studies

Lignans have been attracting the interest of research groups for years. This is due to their phytoestrogenic properties, wide range of biological activities, including antioxidant affect, and availability in various plant species. The basic lignans found in plants are matairesinol, secoisolariciresinol, lariciresinol, pinoresinol, enterolactone and enterodiol, which show estrogenic activity in humans [1,2].

Carbonic anhydrase (CA, EC 4.2.1.1) is a metalloenzyme, which catalyses reversibly hydration of CO_2 and dehydration of HCO_3^- [3-5]. CA first purified from bovine erythrocytes [6,7], are found in tissues such as brain, lung, stomach, pancreas, liver, kidney, muscle tissue, and especially red blood cells [8,9]. It is one of the most important buffer systems in human body. CA plays a crucial role in many physiological events such as acid-base balance, ion exchange, regulation of cardiovascular

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system, and dissolve, transport and dispose of CO_2 in water during respiration [10]. CAs occur in all organisms and, so far, seven different genetic families of CA are known, i.e. α-, β-, γ-, δ-, ζ-, η- and θ-CAs [11]. It has been determined that the average molecular mass of enzymes in mammals is around 30 kDa and sixteen isoenzymes have been identified so far [12]. For the CA enzymes, a wide range of isozymes is present in living systems, depending on the circumstances and requirements of the environment in which they are exist [13].

Cholinesterases are enzymes that have a wide range of distribution in both cholinergic and non-cholinergic tissues, found in plasma and other body fluids [14,15]. Cholinesterases are treated in two groups, (i) acetylcholinesterase (AChE; E.C. 3.1.1.7) and (ii) butyrylcholinesterase (BChE; E.C. 3.1.1.8) [16,17]. AChE is found in grey matter of brain, nerve endings, intestines, lungs, spleen and erythrocytes in high concentrations. Moreover, BChE, which is particularly common in animal kingdom, is present in serum, heart, pancreas, liver and brain [18]. Alzheimer's disease (AD), which is a type of dementia, is a common neurodegenerative disorder. This kind of dementia is defined as an insufficiency of functions on memory in brain. One of the most important causes of AD is decrease of acetylcholine in brain [19].

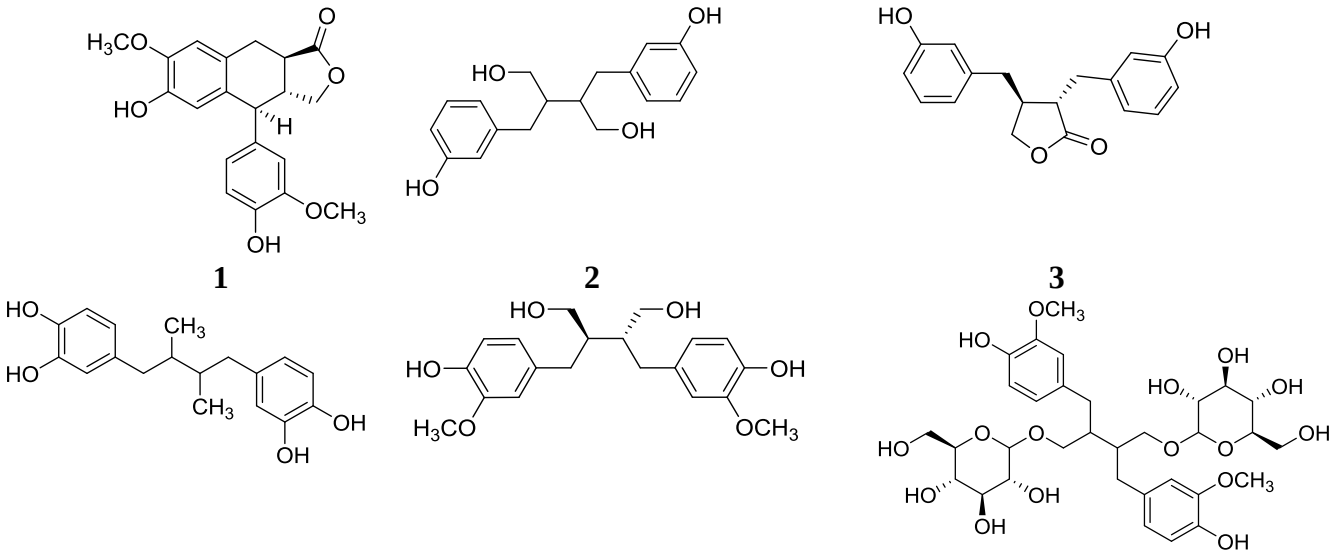

Figure 1. Chemical structures studied lignans **1**-**6**

2. Present Study

CA Activity Assay: Fresh human blood erythrocytes for carbonic anhydrase I, and II isoenzymes were purified using Sepharose-4B-LTyrosine-sulfanilamide affinity chromatography technique [20]. CA activities of the isoenzymes were determined by spectrophotometric measurements at 348 nm [21]. A detailed information and the principal of the method were given in the supporting information (Supporting information S2, Figure S1).

Cholinergic Enzymes Assay: The effects of lignan on AChE / BChE enzymes were determined applying the method developed by Ellman et al., for which AChI and BChI were used as substrates [8,11,22]. For the measurement of activities of both enzymes, 5,5'-dithio-bis(2-nitro-benzoic) acid (DTNB) was used. Moreover, DTNB was also used for the measurement of absorbance of the coloured compound at 412 nm [23]. A detailed information and the principal of the method were given in the supporting information (Supporting information S3, Figure S2).

CA, which also functions as a pH regulator and contains Zn^{2+} metal in the active centre, catalyses the transformation reactions of CO_2 and water to HCO_3^- and H^+ at a very rapid rate using PNA as a substrate [24]. Additionally, CA enzymes take part in vital functions such as diversity and workability [7-10]. For the CA I isoenzyme, IC_{50} values were found as 9.08 nM (r^2:0.9952) for α-(-)-conidendrin, 14.07 nM (r^2:0.9961) for enterodiole, 130.00 nM (r^2:0.9945) for enterolactone, 17.40 nM (r^2:0.9988) for nordihydroguaiaretic acid, 180.00 nM (r^2:0.9994) for secoisolariciresinol and 7.74 nM (r^2:0.9966) for

secoisolariciresinol diglucoside. The positive control acetazolamide showed IC_{50} value of 10.58 nM for the CA I. For the CA II isoenzyme, IC_{50} values were determined to be 17.47nM (r^2:0.9912) for α-(-)-conidendrin, 22.78 nM (r^2:0.9995) for enterodiole, 26.05 nM (r^2:0.9999) for enterolactone, 36.29 nM (r^2:0.9977) for nordihydroguaiaretic acid, 8.96 nM (r^2:0.9916) for secoisolariciresinol and 48.54 nM (r^2:0.9992) for secoisolariciresinol diglucoside. IC_{50} value of the positive control acetazolamide was recorded as 36.85 nM for the CA II. Regarding the Ki values of the tested compounds (**1-6**) and the positive controls (Table 1), remarkable activities against CA I, and CA II in the ranges of 1.27-3.30 nM and 1.1-2.68 nM, respectively, were obtained.

Cholinesterases are enzymes with important functions in many tissues, plasma and body fluids. They are divided into two groups, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), according to their substrate specificity and inhibitor sensitivities [23-25].

IC_{50} values of AChE were found as 8.75 nM (r^2:0.9871) for α-(-)-conidendrin, 9.08 nM (r^2:0.9808) for enterodiole, 14.02 nM (r^2:0.9883) for enterolactone, 14.93 nM (r^2:0.9903) for nordihydroguaiaretic acid, 8.30 nM (r^2:0.9893) for secoisolariciresinol and 7.04 nM (r^2:0.9857) for secoisolariciresinol diglucoside. Positive control, acetazolamide, showed IC_{50} value of 7.53 nM for AChE. Regarding BChE, IC_{50} values were found as 0.74 nM (r^2:0.9690) for α-(-)-conidendrin, 0.66 nM (r^2:0.9680) for enterodiole, 0.64 nM (r^2:9884) for enterolactone, 0.78 nM (r^2:0.9875) for nordihydroguaiaretic acid, 0.70 nM (r^2:0.9746) for secoisolariciresinol and 0.36 nM (r^2:0.9709) for secoisolariciresinol diglucoside. The standard compound, tacrine, for BChE, displayed IC_{50} value of 1.24 nM. The results clearly indicated that all the lignans had effective AChE and BChE inhibition effects.

Table 1. The inhibition effects of some phenolic compounds on human carbonic anhydrase I, and II isoforms (hCA I, and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

Compounds	**K_i (nM)**			
	hCA I	**hCA II**	**AChE**	**BChE**
α-(-)-Conidendrin **(1)**	2.11	2.03	1.54	0.15
Enterodiole **(2)**	2.74	2.68	1.41	0.14
Enterolactone **(3)**	3.30	2.63	1.29	0.16
Nordihydroguaiaretic acid **(4)**	2.81	2.56	1.62	0.17
Secoisolariciresinol **(5)**	2.14	2.52	1.30	0.20
Secoisolariciresinol diglucoside **(6)**	1.27	1.11	0.72	0.08
Acetazolamide*	4.03	2.90	-	-
Tacrine**	-	-	2.90	0.26

*Acetazolamide was used as positive control for human carbonic anhydrase I, and II isoforms (hCA I, and II)
**Tacrine was used as positive control for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes

In conclusion, lignans, as an important group of natural compounds, showed strong inhibition effects toward the hCA I, and II, AChE and BChE enzymes. α-(-)-conidendrin (**1**), enterodiole (**2**), enterolactone (**3**), nordihydroguaiaretic acid (**4**), secoisolariciresinol (**5**) and secoisolariciresinol diglucoside (**6**) could be considered as potential drug candidates for treatment of some diseases such as glaucoma, mountain sickness, epilepsy, gastric and duodenal ulcers, and neurological disorders.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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