**Supporting Information**

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

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## S1. Chemicals

α-(-)-conidendrin (54297-10MG), enterodiole (45198-5MG-F), enterolactone (45199-5MG-F), nordihydroguaiaretic acid (74540-1G), secoisolariciresinol (60372-5MG-F), and secoisolariciresinol diglucoside (S0202-10MG) were purchased from Sigma-Aldrich Co. (St. Luis, USA).

## S2. CA Activity Assay

The method is based on the fact that CA has esterase activity. Principle of method; p-nitrophenylacetate (PNA) used as the substrate of the carbonic anhydrase enzyme hydrolysed p-nitrophenolate, which had maximum absorbance at 348 nm. The reaction mechanism is as follows [1].

After the reaction medium was prepared using a 1 mL quartz cuvette, the difference between the absorbance value at 348 nm at 25 ° C and the absorbance value at the zero second and at end of the third minutes was taken. The $IC_{50}$ values were obtained from activity (%) towards lignan molecules concentration plots [2-3]. In this study were studied five different concentration ranges for the PNA used as the substrate. Then, to determine the Ki values of the CA isoenzymes were used three different concentration ranges of each lignan molecule. The Ki values also represent a numerical value of the affinity of the lignan molecules to both isoenzymes. After all, a Lineweaver-Burk chart was drawn for the inhibitors [4].

The Bradford method was used for the quantitative determination of the protein [5]. This method is based on the protein binding of Coomassie Brilliant Blue G-250. The complex formed by the interaction of the luminescent material and the protein shows the maximum absorbance at 595 nm [6]. Bovine serum albumin was used also as standard for this assay [7].

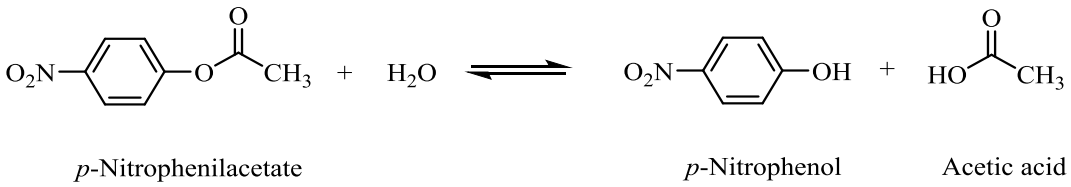
After purification of hCA I, and II isoenzymes, the purity of the enzymes was checked by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), a method developed by Laemmli [8] as described previously in details [9,10]. SDS-PAGE technique was used after purification of the CA isoenzymes was performed. To accomplish this process, to obtain a Minigel system (Mini-PROTEAN Tetra System) was used a sorting gel containing acrylamide (10% and 3%) and SDS (0.1%) [11]. Samples were stained with Coomassie Brilliant Blues R-250 after electrophoresis on a 20% separation gel [12].

## S3. Cholinergic Enzymes Assay

To study the effects of lignan compounds on AChE / BChE enzymes was used the method developed by Ellman et al. [13] as described in previous studies [14,15]. In this method, AChI and BChI were used as substrates of the enzymes. For the measurement of both enzyme activities, 5,5'-dithio-bis (2-nitro-benzoic) acid (DTNB, D8130-1G, Sigma-Aldrich, Sternheim, Germany) was used. DTNB also provided for absorbance measurement at 412 nm by performing colour compound formation.

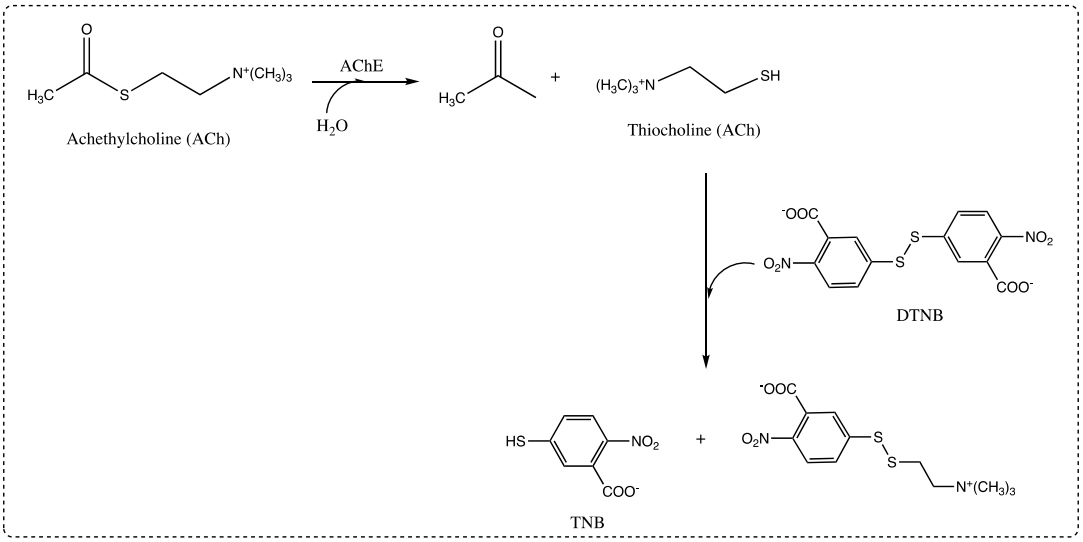
In other words, 10 mL of sample solutions and 100 mL of buffer (Tris/HCl, 1 M, pH 8.0) are mixed at different concentrations using deionized water. Then, 50 mL of AChE / BChE (5.3210-3 EU) was added to the mixture medium and incubated at 25 °C for 10 minutes. After incubation, the reaction was started by the addition of 50 mL DTNB (0.5 mM) and finally 50 mL AChI/BChI. The enzymatic hydrolysis of AChI / BChI results in the formation of thiocholine. The resulting thiocholine also attacks the DTNB nucleophilically and the reaction is spectrophotometrically calculated by virtue of the formation of the 5-thio-2-nitrobenzoate anion, which is yellow in colour and absorbs at 412 nm. The $IC_{50}$ values were acquired from activity (%) towards lignan molecules concentration plots [16]. For establishing of Ki constants in the media with lignan molecules [17,18] using as inhibitor, the varied ACh/BCh concentrations were used as substrates.

**S4. Figure S1.** Hydrolysis mechanism of carbonic anhydrase using *p*-Nitrophenylacetate (PNA) as substrate




*p*-Nitrophenilacetate *p*-Nitrophenol Acetic acid

**S5. Figure S2.** Hydrolysis mechanism of acetylcholinesterase anhydrase acetylcholine (ACh) as substrate




## References


[1] M. Güney, A. Coşkun, F. Topal, A. Daştan, İ. Gülçin and C.T. Supuran (2014). Oxidation of cyanobenzocycloheptatrienes: Synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives, *Bioorg. Med. Chem.* **22**, 3537-3543.

[2] S. Göksu, A. Naderi, Y. Akbaba, P. Kalın, A. Akıncıoğlu, İ. Gulcin, S. Durdağı and R.E. Salmas (2014). Carbonic anhydrase inhibitory properties of novel benzylsulfamides using molecular modeling and experimental studies, *Bioorg. Chem*. **56**, 75-82.

[3] Y. Akbaba, E. Bastem, F. Topal, İ. Gülçin, A. Maraş and S. Göksu (2014). Synthesis and carbonic anhydrase inhibitory effects of novel sulfamides derived from 1-aminoindanes and anilines, *Arch. Pharm*. **347**, 950-957.

[4] B. Arabaci, İ. Gülçin, and S. Alwasel (2014). Capsaicin: A potent inhibitor of carbonic anhydrase isoenzymes, *Molecules* **19**, 10103-10114.

[5] H. Lineweaver and D. Burk (1934). The determination of enzyme dissociation constants, *J. Am. Chem.* Soc. **56**, 658-666.

[6] M.M. Bradford (1976). A rapid and sensitive method for the quantitation of protein utilizing the principle of protein dye binding, *Anal. Biochem.* **72**, 248-254.

[7] B. Aydin, I. Gülcin and S.H. Alwasel (2015). Purification and characterization of polyphenol oxidase from Hemşin apple (*Malus communis* L.), *Int. J. Food Propert.* **18**, 2735–2745.

[8] Z. Huyut, S. Beydemir and İ. Gülçin (2016). In vitro and in vivo inhibitory effects of some phenolic compounds on the activities of carbonic anhydrase, *J. Enzyme Inhib. Med. Chem.* **31**, 1234-1240.

[9] D.K. Laemmli (1970). Cleavage of structural proteins during ın assembly of the head of bacteriophage T4, *Nature*. **227**, 680-685.

[10] E. Mete, B. Comez, H.I. Gul, İ. Gülçin and C.T. Supuran (2016). Synthesis and carbonic anhydrase inhibitory activities of new thienyl substituted pyrazoline benzensulfonamides, *J. Enzyme Inhib. Med.* Chem. **31**, 1-5.
[11] H. Göcer, A. Akıncıoğlu, S. Göksu and I. Gülçin (2017). Carbonic anhydrase inhibitory properties of phenolic sulfonamides derived from dopamine related compounds, *Arab. J. Chem.* **10**, 398-402.
[12] E. Köksal and İ. Gülçin (2008). Purification and characterization of peroxidase from cauliflower (Brassica oleracea L.) buds, *Protein Peptide Lett.* **15**, 320–326.
[13] T.A. Çoban, Ş. Beydemir, İ. Gülçin and D. Ekinci (2008). The inhibitory effect of ethanol on carbonic anhydrase isoenzymes: in vivo and in vitro studies, *J. Enzyme Inhib. Med. Chem.* **23**, 266-270.
[14] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherston (1961). A New and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7**, 88-95.
[15] D. Ozmen Ozgun, C. Yamali, H.İ. Gül, P. Taslimi, İ. Gülçin, T. Yanik and C.T. Supuran (2016). Inhibitory effects of isatin mannich bases on carbonic anhydrases, acetylcholinesterase and butyrylcholinesterase, *J. Enzyme Inhib. Med. Chem.* **31**, 1498-1501.
[16] A. Sujayev, E. Garibov, P. Taslimi, İ. Gülçin, S. Gojayeva, V. Farzaliyev, S.H. Alwasel and C.T. Supuran (2016). Synthesis of some tetrahydropyrimidine-5-carboxylates, determination of their metal chelating effects and inhibition profiles against acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase, *J. Enzyme Inhib. Med. Chem.* **31**, 1531-1539.
[17] P. Taslimi, A. Sujayev, S. Mamedova, P. Kalın, İ. Gulcin, N. Sadeghian, S. Beydemir, Ö.İ. Küfrevioglu, S.H. Alwasel, V. Farzaliyev and S. Mamedov (2017). Synthesis and bioactivity of several new hetaryl sulphonamides, *J. Enzyme Inhib. Med. Chem.* **32**, 137-145.
[18] M. Şentürk, İ. Gülçin, Ş. Beydemir, Ö.İ. Küfrevioğlu and C.T. Supuran (2011). In vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds, *Chem. Biol. Drugs Des.* **77**, 494-499.