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ORIGINAL ARTICLE

Antipsychotic agents screened as human carbonic anhydrase I and II inhibitors

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Abstract

The antipsychotic drugs currently used to treat schizophrenia can be divided into two distinct classes, *typical* and *atypical* antipsychotics. Many drug molecules are enzyme inhibitors that bind reversibly or irreversibly to their target through intermolecular interactions. That's why enzyme inhibition studies are an important issue for drug design and biochemical applications. In this study, *in vitro* inhibition effect of some antipsychotic drugs on the purified carbonic anhydrase (CA) I and II isoenzymes were investigated by using CO_2 as a substrate. CA I and II were purified from human erythrocytes by a simple one step procedure using Sepharose 4B-L-tyrosine-sulfonamide affinity column. The results showed that all the drugs inhibited the cytosolic carbonic anhydrases enzyme activity in a concentration-dependent fashion. Among the studied drugs, aripiprazole and pramipexole were found to be the most active one for hCA I (IC_{50}: 3.64 and 5.37 μM) and hCA II (IC_{50}: 4.16 and 4.81 μM) activity, respectively.

Keywords

Antipsychotic drugs, carbonic anhydrase, inhibition, purification

History

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Introduction

Carbonic anhydrase (EC: 4.2.1.1; CA) is a family of metalloenzymes that catalyse the conversion of CO_2 to HCO_3^- and H^+, being involved in many physiologic processes (Supuran, 2008). CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid-base balance homeostasy, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis, electrolyte secretion, and tumorigenesis among others (Beydemir & Gulcin, 2004; Bottcher *et al*., 1994; Casey, 2006; Hilvo *et al*., 2008; Sly & Hu, 1995; Supuran & Scozzafava, 2002, 2007; Supuran, 2010; Zhenyan *et al*., 2006). Many CA isozymes are well known as therapeutic targets with the potential inhibition or activation effects for the treatment of disorders such as oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis (Casey, 2006; Sly & Hu, 1995; Supuran & Scozzafava, 2002; Supuran, 2010). CA which is a widespread metallo-enzyme has previously been purified and characterized from many living organisms including animals (Beydemir & Gulcin, 2004; Bottcher *et al*., 1994; Zhenyan *et al*., 2006). The isozymes of CA play important roles in different tissues (Bulbul *et al*., 2003; Supuran *et al*., 2001). The similarities of CAs from various sources have been determined from their crystal structures (Huang *et al*., 1998). It is known that carbonic anhydrase has been purified many times from different organisms and the affects of various chemicals, pesticides and drugs on its activity have been investigated (Arslan *et al*., 2011; Celik *et al*., 1996; Coban *et al*., 2009; Ekinci *et al*., 2007; Gervais & Tufts, 1999; Senturk *et al*., 2011, 2012; Vitale *et al*., 1996).

The antipsychotic drugs currently used to treat schizophrenia can be divided into two distinct classes, *typical* or first generation antipsychotics (FGAs) and *atypical* or second generation antipsychotics (SGAs) (Carpenter *et al*., 1998; Miyamoto *et al*., 2005; Sawa & Snyder, 2002). The distinction between these two drug classes is based on the time of introduction to the market, FGAs preceding SGAs, and their receptor binding profiles. FGAs block DA D2 receptors, while SGAs have antagonist activity at both D2 and $5HT_2$ receptors (Creese *et al*., 1976; Richtand *et al*., 2007; Roth *et al*., 2004; Seeman *et al*., 1976). Of greatest importance, however, is the ability, albeit limited, of SGAs to treat the negative symptoms of schizophrenia that is coupled with a lower risk of developing the tardive dyskinesias associated with FGA use (Carpenter *et al*., 1998; Miyamoto *et al*., 2005; Sawa & Snyder, 2002).

Our groups recently investigated the interaction of two mammalian CA isozymes with several compounds, such as analgesic drugs and a series of anabolic compounds, pesticides, macro cyclic thiocrown ethers, cattle drugs and coumarin derivatives (Arslan *et al*., 2012; Cicek *et al*., 2012; Gençer *et al*., 2012a, b; Gokce *et al*., 2012; Karatas *et al*., 2013).

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In literature, there are so many inhibition studies of CA isozymes with sulphonamides compounds, but there are not enough studies related to the inhibition of CA isozymes, especially by antipsychotic drugs. In this present study, we tried to examine the *in vitro* inhibitory effects of 25 antipsychotic drugs on the purified cytosolic CA I and II isoenzymes from human erythrocytes.

Materials and methods

Materials

Sepharose 4B, L-tyrosine, sulphonamide, protein assay reagents and chemicals for electrophoresis were obtained from Sigma Chemical Co. All other chemicals used were of analytical grade and obtained from either Sigma or Merck. All drugs were provided by the local pharmacy.

CA enzyme assay

Cytosolic CA I and II isozymes were purified from human erythrocytes by a simple one step procedure using Sepharose 4B-L-tyrosine-sulphonamide affinity column (Arslan *et al.*, 1996). CA activity was measured based on the determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration as described by Maren (1960). The assay solution was 0.5 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 10.0) and Phenol Red was added as the pH indicator. CO_2-hydratase activity (enzyme units (EU)) was calculated by using the equation t_0–tc/tc where t_0 and tc are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

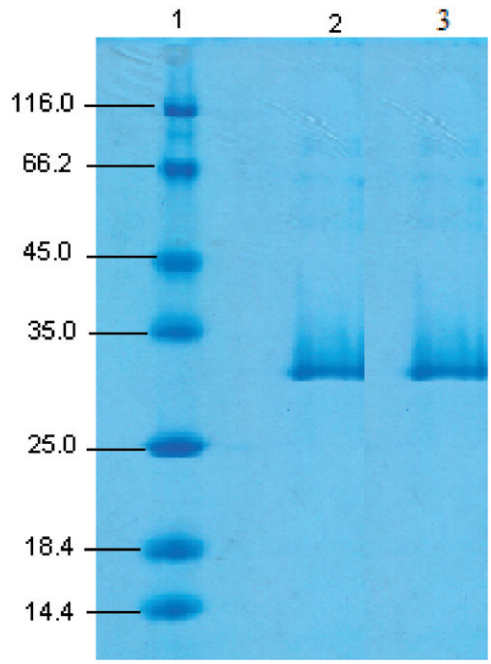


Figure 1. SDS-PAGE of human carbonic anhydrase isozymes. The poled fractions from affinity chromatography (Sepharose 4-B, L-tyrosine, sulphonilamide) was analysed by SDS-PAGE (%12 and %3) and revealed by Coomassie Blue staining. Experimental conditions were as described in the method. Lane 1 contained 5 µg of various molecular mass standards: β-galactosidase (116.0), bovine serum albumin (66.2), ovalbumin (45.0), lactate dehydrogenase (35.0), Restriction endonuclease (25.0), β-lactoglobulin (18.4), lysozyme (14.4). 100 microgram of purified human carbonic anhydrase I and II (lane 2 and lane 3) migrated with a mobility corresponding to an apparent Mr 33.0 kDa.

In vitro inhibition studies

For the inhibition studies of antipsychotic drugs, different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each drug were determined by regression analysis using Microsoft Office 2000 Excel. CA enzyme activity without a drug solution was accepted to be 100%.

Preparation of hemolysate and purification of CA isozymes from human red blood cells

Blood samples (25 mL) were taken from healthy human volunteers. The blood samples were anti-coagulated with acid-citrate-dextrose, centrifuged at 5000 rpm for 10 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then hemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 15 000 rpm for 30 min at 4 °C

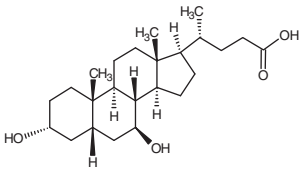
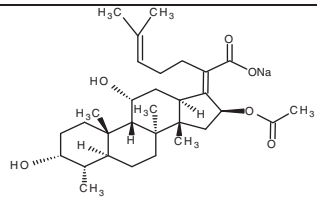
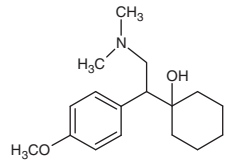
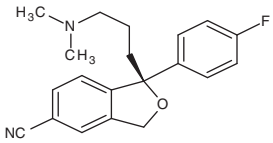
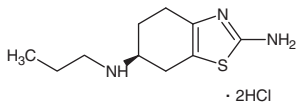
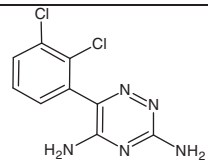
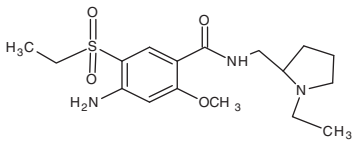
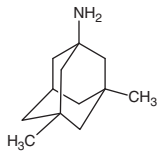
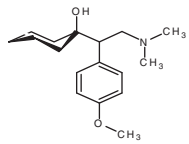
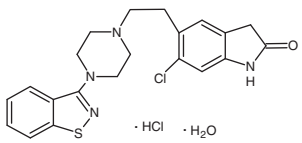
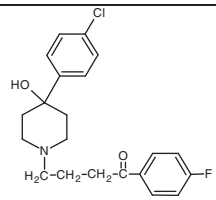
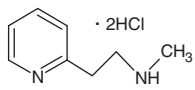
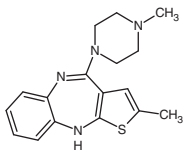
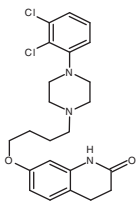
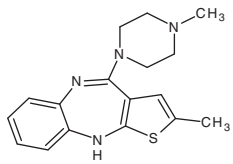
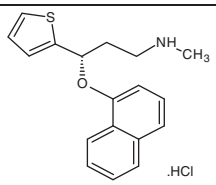
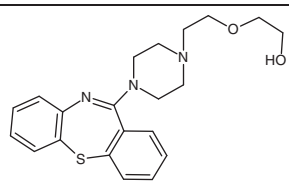
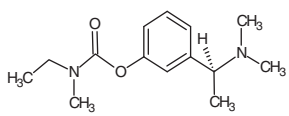
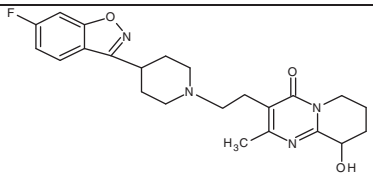
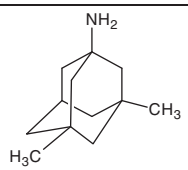
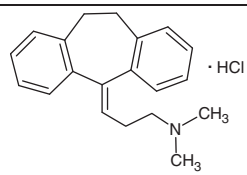
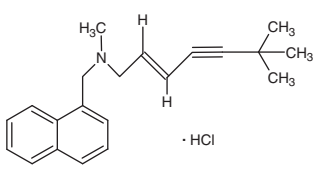
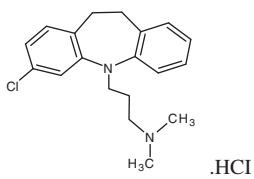
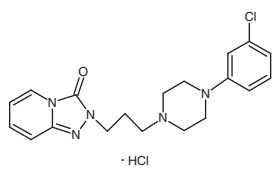
Table 2. The IC_{50} values of antipsychotic drugs.

No.	Drug substance	hCA I IC_{50} (µM)	hCA II IC_{50} (µM)
1	Memantine hydrochloride	132.92	142.93
2	Clomipramine hydrochloride	247.94	278.36
3	Amitriptyline hydrochloride	205.75	260.34
4	Lamotrigine	811.20	977.30
5	Venlafaxine	596.71	742.53
6	Sodium fusidate	3868.00	3390.00
7	Haloperidole	95.69	139.58
8	Duloxetine hydrochloride	536.00	278.35
9	Ursodeoxycholic acid	2067.00	2329.00
10	Rivastigmine	28.25	27.71
11	Pramipexole	5.37	4.81
12	Olanzapine	131.59	119.61
13	Paliperidone	61.10	94.51
14	Escitalopram	420.10	637.25
15	Betahistine dihydrochloride	376.13	327.81
16	Escitalopram	240.66	290.92
17	Aripiprazole	3.64	4.16
18	Trazodone hydrochloride	308.63	393.58
19	Terbinafine hydrochloride	2867.00	3288.00
20	Ziprasidone	315.64	385.41
21	Amisulpride	503.78	1536.00
22	Olanzapine	224.88	301.13
23	Memantine	158.49	197.75
24	Quetiapine	1472.00	1778.00
25	Venlafaxine	476.63	528.54

Table 1. Summary of the purification of human carbonic anhydrase I and II.

Step	Volume (ml)	Activity (U/ml)	Total activity (U)	Protein amount (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Overall yield %	Overall purification (fold)
Hemolysate	25	41.33	1033.25	1.9980	49.9500	20.68	100.00	–
Affinity chromatography	2	83.08	166.16	0.0153	0.0306	5430.06	16.08	262.57

Table 3. Chemical structures of antipsychotic drugs.

 Ursodeoxycholic acid	 Sodium fusidate	 Venlafaxine
 Escitalopram	 Pramipexole	 Lamotrigine
 Amisulpride	 Memantine	 Venlafaxine
 Ziprasidone	 Haloperidole	 Betahistine dihydrochloride
 Olanzapine	 Aripiprazole	 Olanzapine
 Duloksetin hydrochloride	 Quetiapine	 Rivastigmine
 Paliperidone	 Memantine hydrochloride	 Amitriptyline HCI
 Terbinafine hydrochloride	 Clomipramine HCI	 Trazodone hydrochloride

and the pH of the hemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL hemolysate was applied to the affinity column comprising Sepharose-4B-L-tyrosine-sulfonamide gel equilibrated with 25 mM Tris-HCl/0.1 M Na_2SO_4 (pH 8.5) (Arslan *et al*., 1996). The affinity gel was washed with 50 mL of 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.5). The hCA isozymes were then eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), which recovered hCA-I and hCA-II respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

Results and discussion

In this study, CA I and II isozymes from human erythrocytes were purified by a simple one step procedure using Sepharose 4B-L-tirozin-sulfonamide affinity column and purity of the enzyme was confirmed by SDS–PAGE (Figure 1). The overall purification gave CA in a yield of 16.08% with a specific activity of 5430.06 EU/mg proteins and the overall purification was 262.57-fold (Table 1). Results are listed in Table 2, in terms of molarity of the tested drugs causing a 50% reduction of the enzymatic activities. The results showed that all the drugs inhibited the enzyme activity in a concentration-dependent fashion. The inhibition values against CAs were given in the Table 2. It is determined that the inhibition values are in between 5.37–3868.00 μM for hCA I and 4.81–3390.00 μM for hCA II. As shown in Table 2, aripiprazole and pramipexole were found to be the most active one for hCA I (IC_{50}: 3.64 and 5.37 μM) and hCA II (IC_{50}: 4.16 and 4.81 μM) activity, respectively.

In literature, it has been reported that IC_{50} values of sodium ampicillin were 0.385 mM on hCA-I and 0.774 mM on hCA-II (Beydemir *et al*., 2000). Ozensoy *et al*. (2008) reported that cefuroxime axetil for hCA I and hCA II isozymes as IC_{50} value of 2.92×10^{-5} and 2.12×10^{-5} mM, respectively. Recently, our group determined that the IC_{50} concentrations of dexketoprofen trometamol and dexamethasone sodium phosphate on hCA I were 683 and 4250 μM and for hCA II 950 and 6200 μM respectively (Gokce *et al*., 2012).

Puscas *et al*. (2001) reported that indomethacin, *in vitro* and *in vivo*, induces an increase in erythrocyte CA I and CA II activity. In humans, an increase or decrease in erythrocyte CA II activity is correlated with an increase or decrease in gastric acid secretion. Indomethacin is not only an activator of CA but also antagonizes the affect of acetazolamide, a specific inhibitor of this enzyme. Many drug side effects may be considered to result from CA isozyme inhibition. For example, respiratory acidosis is probably the cause of some side effects observed during acetazolamide therapy, such as fatigue, headache, altered taste sensations and distress (Thomsen *et al*., 2000). Measurement of the CO_2 hydratase activity of CA-I and CA-II requires specific inhibitors or separation of the isozymes. It is difficult to study the factors and conditions that affect CA activity because standard CA activity assays have serious limitations. Therefore, estimate of the CA-I and CA-II level in erythrocytes are complicated by the pronounced differences in enzymatic activity of CA-I and CA-II (Nishita *et al*., 2005).

Many drug molecules are enzyme inhibitors that bind reversibly or irreversibly to their target through intermolecular interactions. That is why enzyme inhibition studies are an important issue for drug design and biochemical applications (Alim & Beydemir, 2012; Demir *et al*, 2012a, b; Sayin *et al*., 2012; Sonmez *et al*., 2011). This study is the first report on the inhibition of cytosolic carbonic anhydrases by various antipsychotic drugs. Among the studied drugs, at low concentrations, aripiprazole and pramipexole had the strongest *in vitro* inhibitory effects on hCA I (IC_{50}: 3.64 and 5.37 μM) and hCA II (IC_{50}: 4.16 and 4.81 μM) activity. As stated earlier, many drug side effects may be considered to result from CA isozyme inhibition. Uncontrolled usage of these mentioned drugs could cause serious adverse effects and could be deleterious to health. For this reason, these drugs must be used carefully and the dosage should be closely monitored to decrease their side effects. Depending on our data, it is understood that usage of these drugs must be well-determined as they might have serious side effects on CA enzymes which may result in the disruption of acid-base balance and salt transport. Consequently, including the outcome of this present study results, further *in vivo* studies could help to reveal the inhibition mode of these drugs on cytosolic carbonic anhydrases. Detailed information regarding the structure of all drugs presented in this letter can be found in the supplementary data provided (as shown in Table 3).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References

Alim Z, Beydemir S. (2012). Effects of some anti-neoplastic drugs on sheep liver sorbitol dehydrogenase. *Arch Physiol Biochem*, 118, 244–52.

Arslan M, Beyaztas S, Erzengin M. (2011). *In vitro* effects of some antibiotics on enzyme activity of carbonic anhydrase from bovine erythrocytes. *Fresenius Env Bul*, 20:439–45.

Arslan M, Gençer N, Arslan O, Guler OO. (2012). In vitro efficacy of some cattle drugs on bovine serum paraoxonase 1 (PON1) activity. *J Enzyme Inhib Med Chem*, 27:722–9.

Arslan O, Nalbantoglu B, Demir N, *et al*. (1996). A new method for the purification of carbonic anhydrase isozymes by affinity chromatography. *Turk J Med Sci*, 26:163–6.

Beydemir S, Ciftci M, Ozmen I, *et al*. (2000). Effects of some medical drugs on enzyme activities of arbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. *Pharma Res*, 42:187–91.

Ekinci D, Beydemir S, Alim Z. (2007). Some drugs inhibit *in vitro* hydratase and esterase activities of human carbonic anhydrase-I and II. *Phar Rep*, 59:580–7.

Beydemir S, Gulcin I. (2004). I. Affects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. *J Enzyme Inhib Med Chem*, 19:193–7.

Bottcher K, Waheed A, Sly WS. (1994). Membrane-associated carbonic anhydrase from the crab gill: Purification, characterization, and comparison with mammalian CAs. *Arch Biochem Biophys*, 312:429–35.

Bulbul M, Hisar O, Beydemir S, *et al*. (2003). The in vitro and in vivo inhibitory affects of some sulfonamide derivatives on rainbow trout (Oncorhynchus Mykiss) erythrocyte carbonic anhydrase activity. *J Enz Inhib Med Chem*, 18:371–5.

Casey JR. (2006). Why bicarbonate. *Biochem Cell Biol*, 84:930–9.

Celik I, Camas H, Arslan O, Kufrevioglu OI. (1996). The affects of some pesticides on human and bovine erythrocyte carbonic anhydrase enzyme activities in vitro. *J Envıron Sci Heal A*, 31:2651–7.

Cicek B, Ergun A, Gençer N. (2012). Synthesis and evaluation in vitro effects of some macro cyclic thiocrown ethers on erythrocyte carbonic anhydrase I and II. *Asian J Chem*, 24:3729–31.

Coban TA, Beydemir S, Gulcin I, *et al*. (2009). Sildenafil is a strong activator of mammalian carbonic anhydrase isoforms I–XIV. *Bio Med Chem*, 17:5791–5.

Creese I, Burt DR, Snyder SH. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192:481–3.

Demir D, Gençer N, Er A. (2012a). Purification and characterization of prophenoloxidase from Galleria mellonella L. *Artif Cells Blood Substit Immobil Biotechnol*, 40:391–5.

Demir D, Gençer N, Arslan O, *et al*. (2012b). In vitro inhibition of polyphenol oxidase by some new diarylureas. *J Enzyme Inhib Med Chem*, 27:125–31.

Gençer N, Ergun A, Demir D. (2012a). In vitro effects of some anabolic compounds on erythrocyte carbonic anhydrase I and II. *J Enzyme Inhib Med Chem*, 27:208–10.

Gençer N, Ergun A, Demir D. (2012b). In vitro effects of some pesticides on human erythrocyte carbonic anhydrase activity. *Fresen Envir Bullet*, 21:549–52.

Gervais MR, Tufts BL. (1999). Characterization of carbonic anhydrase and anion exchange in the erythrocytes of bowfin (Amia calva), a primitive air-breathing fish. *Comp Biochem Phys*, 23A:343–50.

Gokce B, Gençer N, Arslan O, *et al*. (2012). Evaluation of in vitro effects of some analgesic drugs on erythrocyte and recombinant carbonic anhydrase I and II. *J Enzyme Inhib Med Chem*, 27:37–42.

Hilvo M, Baranauskiene L, Salzano AM, *et al*. (2008). Biochemical characterization of CA IX, one of the most active carbonic anhydrase isozymes. *J Biol Chem*, 283:27799–809.

Huang S, Xue Y, Sauer-Eriksson E, *et al*. (1998). Crystal structure of carbonic anhydrase from Neisseria gonorrhoeae and its complex with the inhibitor acetazolamide. *J Mol Biol*, 283:301–10.

Karatas MO, Alici B, Cakir U, *et al*. (2013). Synthesis and carbonic anhydrase inhibitory properties of novel coumarin derivatives. *J Enzyme Inhib Med Chem*, 28:317–22.

Maren TH. (1960). A simplified micromethod for the determination of carbonic anhydrase and its inhibitors. *J Pharm Exp Ther*, 130:2629–34.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA. (2005). Treatments for schizophrenia: A critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry*, 10:79–104.

Nishita T, Takahasi M, Kasuya T, *et al*. (2005). Measurement of erythrocyte carbonic anhydrase isozymes (CA-I and CA-II) in racehorses and riding horses. *J Vet Med Sci*, 67:63–7.

Ozensoy O, Arslan O, Kockar F. (2008). Differential in vitro inhibition effects of some antibiotics on tumor associated carbonic anhydrase isozymes of hCA-IX and hCA-XII. *J Enzyme Inhib Med Chem*, 23:579–85.

Puscas I, Ifrim M, Maghiar T, *et al*. (2001). Indomethacin activates carbonic anhydrase and antagonizes the affect of the specific carbonic anhydrase inhibitor acetazolamide, by a direct mechanism of action. *Inter J Clinic Pharm Therap*, 39:265–70.

Richtand NM, Welge JA, Logue AD, *et al*. (2007). Dopamine and serotonin receptor binding and antipsychotic efficacy. *Neuropsychopharmacology*, 32:1715–26.

Roth BL, Sheffler DJ, Kroeze WK. (2004). Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discovery*, 3:353–9.

Sayin D, Cakir DT, Gençer N, Arslan O. (2012). Effects of some metals on Paraoxonase activity from shark Scyliorhinus canicula. *J Enzyme Inhib Med Chem*, 27:595–8.

Sawa A, Snyder SH. (2002). Schizophrenia: Diverse approaches to a complex disease. *Science*, 296:692–5.

Seeman P, Lee T, Chau-Wong M, Wong K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature*, 261:717–19.

Senturk M, Gulcin I, Beydemir S, *et al*. (2011). *In vitro* inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. *Chem Biol Drug Des*, 77:494–9.

Senturk M, Alıcı HA, Beydemir S, Kufrevioglu OI. (2012). *In vitro* and *in vivo* effects of some benzodiazepine drugs on human and rabbit erythrocyte carbonic anhydrase enzymes. *J Enzyme Inh Med Chem*, 27:680–4.

Sly WS, Hu PY. (1995). Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu Rev Biochem*, 64:375–401.

Supuran CT. (2008). Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nature Rev Drug Discov*, 7:168–81.

Supuran CT. (2010). Carbonic anhydrase inhibitors. *Bioorg Med Chem Lett*, 20:3467–74.

Supuran CT, Scozzafava A. (2007). Carbonic anhydrases as targets for medicinal chemistry. *Bioorg Med Chem*, 15:4336–50.

Supuran CT, Scozzafava A. (2002). Applications of carbonic anhydrase inhibitors and activators in therapy. *Expert Opin Ther Pat*, 12:217–42.

Supuran CT, Briganti F, Tilli S, *et al*. (2001). Carbonic anhydrase inhibitors: Sulfonamides as antitumor agents. *Bioorg Med Chem*, 9:703–14.

Sonmez F, Sevmezler S, Atahan A, *et al*. (2011). Evaluation of new chalcone derivatives as polyphenol oxidase inhibitors. *Bioorg Med Chem Letters*, 21:7479–82.

Thomsen J, Charabi S, Tos M. (2000). Preliminary results of a new delivery system for gentamicin to the inner ear in patients with Meniere's disease. *Eur Arch Otorhinolaryngol*, 257:362–5.

Vitale AM, Monserrat JM, Castilho P, Rodriguez EM. (1999). Inhibitory affects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab Chasmagnathus granulata (Decapoda, Grapsidae). *Comp Biochem Physiol C*, 122:121–9.

Zhenyan Y, Liping X, Seunghwan L, Rongqing ZA. (2006). Novel carbonic anhydrase from the mantle of the pearl oyster (Pinctada fucata). *Comp Biochem Physiol B*, 143:190–4.