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In vitro inhibition effect of some chalcones on erythrocyte carbonic anhydrase I and II

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Abstract

In this study, 4′-(phenylurenyl/thiourenyl)chalcones (14-25) were prepared from 4′-(phenylurenyl/thiourenyl)acetophenones and benzaldehyde derivatives by Claisen-Schmidt condensation. In vitro inhibition effects of chalcone derivatives on purified carbonic anhydrase I and carbonic anhydrase II were investigated by using the CO_2 hydration method of Maren. The result showed that all the synthesized compounds inhibited the CA isoenzymes activity. 18 and 19 were found to be most active (IC50 = 25.41 μM and 23.06 μM) for hCA I, respectively. For hCA II, 24 is the most active compound (IC50 = 14.40 μM).

Keywords: carbonic anhydrase, chalcone, urea, thiourea, inhibition, In vitro

Introduction

Glaucoma is an eye disease in which the optic nerve is damaged related to different risk factors and it can permanently damage vision in the affected eye(s). Elevated intraocular pressure (IOP) is known as the major risk factor in this disease. Therefore, targeting IOP with different pressure lowering agents is the main treatment strategy for this disease (Siesky et al. 2009).

Because IOP depends on the delicate balance between production of aqueous humor by the ciliary body and its drainage through the various pathways, selective activation of the carbonic anhydrase isoenzymes can increase IOP by facilitating aqueous formation and transport through cell membranes, as well as its release into the posterior chamber (Steele et al. 2009).

Together most of CA isoenzymes are related to treatment of glaucoma, they also involved in these processes which are important therapeutic targets with the potential to be inhibited to treat a range of disorders including edema, obesity, cancer, epilepsy and osteoporosis (Ashida and Brey 1995, Vullo et al. 2003, Nishimori 2004, Lehtonen et al. 2004, Vullo et al. 2005a, 2005b, Nishimori et al. 2005a, 2005b). Given the physiological importance of the CA, the metabolic impact of chemicals for crop production should receive greater study.

With the involvement of the carbonic anhydrase (CA) enzyme family catalyzing the physiological hydration of CO_2 to yield bicarbonate and a proton in many physiological/pathological processes, development of specific inhibitors has been achieved for clinical application (Thiry et al. 2006, Supuran 2008).

The active site of most CAs contains a zinc ion (Zn^{2+}), which is essential for catalysis. To inhibit this enzyme, it can be interacted with different electron donor agent such as coumarin derivatives (Karatas et al. 2013), a series anabolic compounds (Gencer et al. 2012a, 2012b), some macrocyclic thiocrown ethers (Cicek et al. 2012), some snalgesic drugs (Gokce et al. 2012), some oral contraceptives (Kıranoglu et al. 2007) and pesticides (Gencer et al. 2012a, 2012b).

As these compounds, chalcones (1,3-diaryl-2-propen-1-ones) have been studied widely for their interesting pharmacological properties (Di Carlo et al. 1999) and synthetic importances (Trivedi et al. 2008, Gezegen et al. 2010, Piotrowska et al. 2011) for a long time. In this context, a number of chalcone derivatives containing aromatic rings and unsaturated chain, which is responsible for many biological activities such as antitumoral (Cabrera et al. 2007) anticancer and antioxidant (Anto et al. 1995), chemoprotective (Forejtnikova et al. 2005), antifungal (Lahtchev et al. 2008), antinociceptic (Santos et al. 2008) and antimicrobial (Karaman et al. 2010) activities have been found to inhibit several important enzymes in cellular systems, including xanthine oxidase (Sogawa et al. 1994), aldose reductase (Iwata et al. 1999), heme oxygenase (Lee et al. 2006), protein tyrosine kinase (Yang et al. 2001, Nerya et al. 2004) and quinone reductase (Miranda et al. 2000).

On the other hand it has been reported that some urea derivatives have antiviral (Huang et al. 2004), antiinsecticide (Bassett 2004), antitumor (Sun et al. 2010) and tyrosine kinase inhibitor (Engen et al. 2010) activities. Especially, reactions of 4-aminochalcones with isocyanates give

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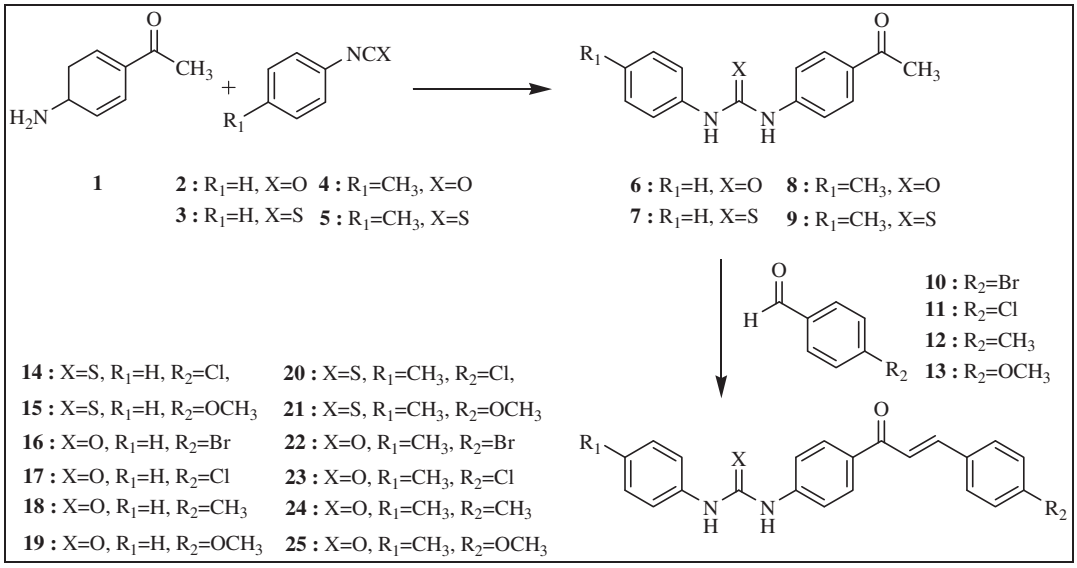

Scheme 1. Synthesis of phenylurea/phenylthiourea-substituted chalcone derivatives.

unsymmetrically substituted urea derivatives which are linked to a series of biological activities including antiglycating (Khan et al. 2009), MCH-R1 antagonists (Galiano et al. 2007), P2Y1 receptor antagonists (Thalji et al. 2010) and heparanase inhibitors (Pan et al. 2006) properties.

Therefore, the investigation of clinically useful chalcones and ureas/thioureas is a growing field of interest. In this study, we describe a number of chalcones containing phenylurea/thiourea groups and study their properties as inhibitors of hCA I and hCA II purified from human erythrocytes.

Materials and methods

Materials

Sepharose 4B, L-tyrosine, sulphanilamide, synthetic starting material, reagents and solvents were of analytical grade and were purchased from Aldrich Chemical Co., Merck Chemical Co. 1-(4-acetylphenyl)-3-phenylureas (**6-9**) and chalcone derivatives (**14-25**) were synthesized according to the literature (Dominguez et al. 2005, Sönmez et al. 2011). All other chemicals used were of analytical grade and obtained from either Sigma or Merck.

General procedure for preparation of 6-9

A mixture of the 4′-aminoacetophenone **1** (10 mmol) and phenylisocyanate or phenylisothiocyanate derivative **2-5** (10 mmol) was dissolved in dry toluene (20 ml). After the mixture was refluxed overnight, dry toluene was added until resulting solid was dissolved, and recrystallization afforded the desired 1-(4-acetylphenyl)-3-phenylurea and thiourea derivatives in pure form. Synthesis of phenylurea/phenylthiourea-substituted chalcone derivatives were prepared according to Scheme 1.

General procedure for preparation of 14-25

Phenylurea or phenylthiourea substituted chalcones **14-25** were synthesized by reacting equimolecular quantities of 1-(4-acetylphenyl)-3-phenylurea or thiourea derivatives **6-9** and the corresponding benzaldehyde derivatives **10-13** in the presence of an excess sodium hydroxide (2.5 mmol) in dry methanol (10 ml) and DMSO (10 ml). After the mixture was stirred overnight at room temperature, cold brine (30 ml) was added to this solution and resulting precipitate was filtered and dried in air. The precipitate was recrystallized from appropriate solvent to give phenylurea or phenylthiourea substituted chalcones **14-25** in pure form.

Preparation of hemolysate and purification from blood red cells

Blood samples (25 ml) were taken from healthy human volunteers. They were anticoagulated with ACD (acid-citrate-dextrose), centrifuged at 2000 × g for 20 min at 4°C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCI and then hemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 2000 × g for 25 min at 4°C, and the pH of the hemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 ml hemolysate was applied to an affinity column containing L-tyrosine-Sulphanilamide -Sepharose-4B (Arslan et al. 1996) equilibrated with 25 mM Tris–HCl/0.1M Na_2SO_4 (pH 8.5). The affinity gel was washed with 50 ml of 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.5). The human CA (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.

CA enzyme assay

Carbonic anhydrase activity was measured by the Maren method which is based on determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration (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 t0-tc/tc where t0 and tc are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively.

In vitro inhibition studies

For the inhibition studies of chalcone different concentrations of these compounds were added to the enzyme. Activity % values of carbonic anhydrase for different concentrations of each chalcone were determined by regression analysis using Microsoft Office 2000 Excel. Carbonic anhydrase enzyme activity without a chalcone solution was accepted as 100% activity.

Results and discussion

All chalcone derivatives (**14-25**) containing phenylurea/thiourea groups were synthesized from the appropriate benzaldehydes (**l0-13**) and acetophenone derivatives (**6-9**) which were obtained by reaction between 4'aminoacetophenone (**1**) and isocyanates/isothiocyanates (**2-5**) according to literature procedures (Dominguez et al. 2005, Sönmez et al. 2011).

In this study, we examined the effects of chalcone derivatives (**14-25**) on hCA I and hCA II. The result showed that all the synthesized compounds inhibited the CA isoenzymes activity. Furthermore, phenylureny/phenylthiourenychalcones containing electron-donating groups generally inhibited hCA I and hCA II isozymes at low IC50 values. The IC50 values of (**14-25**) analogues against hCA I and II were summarized in Table I. Especially **24** inhibited both enzymes with 27.93 μM and 14.40 μM IC50 values. Besides **18** and **19** showed remarkable inhibition effect on hCA I at low IC50, **19** and **25** had inhibition effect at 19.44 and 17.59 μM IC50 values on hCA II.

CA inhibitors lower intraocular pressure by reducing bicarbonate formation in the ciliary process, thus lowering Na^+ transport and flow of aqueous humor: this is the basis for their use in glaucoma treatment. Unfortunately, systemic therapy with parenteral sulphonamides and their derivatives leads to significant side effects, many of them being probably due to inhibition of CA isoforms in other tissues. Acetazolamide is the most widely used inhibitor and has advantages over the others because it is 20 times less active against CAI than against CAII in erythrocytes. But the inhibition of various CA isoforms which present in tissues other than eye leads to an entire range of side effects, the most prominent being numbness and tingling of extremities, metallic taste, depression, fatigue, malaise, weight loss, decreased libido, gastrointestinal irritation, metabolic acidosis, renal calculi and transient myopia (Maren 1960, Arslan et al. 1997, Supuran and Scozzafava 2000). For similar reasons, designing of new drugs is essential for clinical application of treatment of glaucoma.

Table I. The IC_{50} values of chalcone derivatives (**14-25**).

Compounds	hCA I IC_{50} μM	hCA II IC_{50} μM
14	36.26	23.89
15	44.36	30.17
16	29.46	22.54
17	52.25	27.10
18	25.41	27.20
19	23.06	19.44
20	50.79	37.06
21	60.08	41.59
22	36.00	30.66
23	51.90	28.62
24	27.93	14.40
25	34.57	17.59

In summary, enzyme inhibition is more important issue for drug design and biochemical applications (Aydemir and Kavrayan 2009, Demir et al. 2012, Bytyqi-Damoni et al. 2012, Demirel and Tarhan 2004, Senturk et al. 2012). The results showed that new chalcone derivatives inhibited the hCA I and II enzyme activity. Therefore, our results suggested that phenylurenyl/thiourenyl chalcone derivatives are likely to be adopted as candidates to treat glaucoma and may be taken for further evaluation in *in vivo* studies.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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Notice of Correction

This paper published online on 18 January 2013 contained an error in the list of references. The following references were written incorrectly as follows:

Çiçek B, Ergün 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. 24:3729–3731.
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The error has been corrected for this version.