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Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: https://www.tandfonline.com/loi/ienz20

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To cite this article: Fatih Sonmez, Cigdem Bilen, Sinem Sumersan, Nahit Gencer, Semra Isik, Oktay Arslan & Mustafa Kucukislamoglu (2014) *In vitro* inhibition effect and structure–activity relationships of some saccharin derivatives on erythrocyte carbonic anhydrase I and II, Journal of Enzyme Inhibition and Medicinal Chemistry, 29:1, 118-123, DOI: 10.3109/14756366.2012.757222

To link to this article: https://doi.org/10.3109/14756366.2012.757222

Journal of Enzyme Inhibition and Medicinal Chemistry

http://informahealthcare.com/enz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, 2014; 29(1): 118–123
 DOI: 10.3109/14756366.2012.757222

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In vitro inhibition effect and structure–activity relationships of some saccharin derivatives on erythrocyte carbonic anhydrase I and II

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Abstract

In this study, *in vitro* inhibitory effects of some saccharin derivatives on purified carbonic anhydrase I and II were investigated using CO_2 as a substrate. The results showed that all compounds inhibited the hCA I and hCA II enzyme activities. Among the compounds, 6-(*p*-tolylthiourenyl) saccharin (**6m**) was found to be the most active one for hCA I activity ($IC_{50} = 13.67\ \mu M$) and 6-(*m*-methoxyphenylurenyl) saccharin (**6b**) was found to be the most active one for hCA II activity ($IC_{50} = 6.54\ \mu M$). Structure–activity relationships (SARs) study showed that, generally, thiourea derivatives (**6l–v**) inhibited more hCA I and hCA II than urea derivatives (**6a–k**). All compounds (excluding **6c** and **6r)** have higher inhibitory activity on hCA II than on hCA I.

Keywords

Carbonic anhydrase, inhibition, saccharin, thiourea, urea

History

Received 27 September 2012
Revised 6 December 2012
Accepted 6 December 2012
Published online 23 January 2013

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) is a ubiquitous zinc enzyme. Basically, there are several cytosolic forms (CA-I, CA-II, CA-III and CA-VII), four membrane-bound forms (CA-IV, CA-IX, CA-XII and CA-XIV), one mitochondrial form (CA-V), as well as a secreted CA form (CA-VI)[1,2]. They all catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as the gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes[1–3]. CA inhibitors have now been a mainstay of human clinical intervention for several decades, with at least 25 clinically used drugs that are CA inhibitors[4]. Although there are many studies on this enzyme, the CA enzyme family continues to capture the attention of drug discovery scientists and clinicians as the knowledge regarding the therapeutic implications associated with this enzyme class continues to grow[4,5].

Saccharin, 1,2-benzisothiazole-3-one-1,1-dioxide, is a well-known heterocyclic compound and has been used as a sweetener in the form of its sodium salt since 1885. Yet it is also a heterocycle of pharmaceutical importance, being a key structural element of certain CNS-active drugs[6]. Chemically, saccharin consists of a sulfimide with a lactam and cyclic sulfonamide moiety. The latter functionality is responsible for the acidic character of the molecule and suggests its potential to interact with the zinc ion at the floor of the binding pocket of carbonic anhydrases. Köhler et al. presented saccharin as zinc-binding portion based on the X-ray structure[7].

Sulfonamides are the best known inhibitors of CA enzymes and are used for the treatment of glaucoma in medicinal chemistry[8]. Acetazolamide (AAZ), dorzolamide (DZA) and brinzolamide (BRZ) are sulfonamide derivatives and are used in the treatment of glaucoma. However, these drugs have several side effects such as numbness and tingling in the fingers and toes, blurred vision, kidney stones, an increase in urination, upset stomach, dry eye and headache or dizziness[2,9].

There are reports[10,11] of many such aromatic sulfonamides or bis-sulfonamide moieties incorporating urea or thiourea groups as very potent inhibitors against three isozymes, human CA I, human CA II and bovine isozyme CA. A small series of five ureido-substituted benzenesulfonamide derivatives were recently investigated as inhibitors of the cytosolic isoform hCA II by one of these groups. It has been observed that their potency varied between 3.3 and 226 nM, and by means of X-ray crystallography a highly variable orientation of the R-ureido moieties was evidenced when the inhibitor was bound within the enzyme active site[11].

In this study, we evaluated 6-(phenylurenyl/thiourenyl) saccharin derivatives (**6a–v**), synthesized in the previous work[12], effects on hCA I and hCA II purified from human erythrocytes. Additionally, we presented SAR analyses.

Materials and methods

General

Sepharose 4B, L-tyrosine, sulfonamide, synthetic starting material, reagents and solvents were purchased from Merck (Darmstadt, Germany), Alfa Easer (Ward Hill, MA), Sigma-Aldrich (Taufkirchen, Germany) and Fluka (Taufkirchen, Germany).

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General procedure for 6-(phenylurenyl/thiourenyl) saccharin derivatives

Phenylisocyanate or phenylisothiocyanate derivatives (1 mmol) were added to a solution of 6-aminosaccharin (1 mmol) and triethyl amine (1 mL) in dry DMF. The mixture was stirred at room temperature for 12 h and then poured into cold 1 M HCl. The precipitate was filtered and washed with cold water. The crude products were recrystallized from ethanol over 99% purity. The synthetic procedures are depicted in Scheme 1.

Preparation of hemolysate and purification from blood red cells

Preparation of hemolysate and purification from blood red cells made by the literature[13] was presented in supporting information.

CA enzyme assay

CA activity measured by the Maren method[14] was presented in supporting information.

In vitro inhibition studies

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

Results and discussion

Chemistry

The synthetic procedures are depicted in Scheme 1. 6-(Phenylurenyl/thiourenyl) saccharin compounds (**6a–v**) were synthesized from 4-nitrotoluene (**1**) in five steps by known procedures[12].

Biological evaluation of saccharin derivatives for hCA I and hCA II inhibitory activities

For evaluating the hCA I and hCA II inhibitory effects, all compounds were subjected to hCA I and hCA II inhibition assay with CO_2 as a substrate. The result showed that all compounds (**6a–v**) inhibited the hCA I and hCA II enzyme activity.

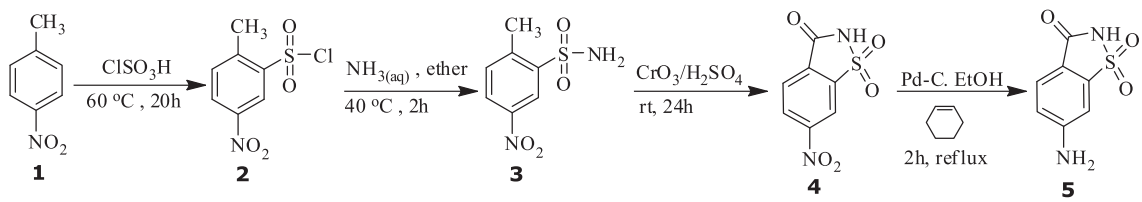
The IC_{50} values and inhibition constants of **6a–v** analogues against hCA I and hCA II are summarized in Table 1 and the IC_{50} graphs are given in Figure 1. The IC_{50} figures are presented as supporting information.

We have determined the IC_{50} values of 13.57–74.90 μM for the inhibition of hCA I and 6.54–49.00 μM for the inhibition of hCA II. Among all compounds, **6m** ($IC_{50} = 13.67$ μM) was found to be the most active one for hCA I inhibitory activity and **6b** ($IC_{50} = 6.54$ μM) showed the highest hCA II inhibitory activity. **6d** ($IC_{50} = 30.81$ μM) was found to be the most active one for hCA I inhibitory activity and **6b** ($IC_{50} = 6.54$ μM) showed the highest hCA II inhibitory activity for the urea derivatives. Among the thiourea derivatives, **6m** ($IC_{50} = 13.67$ μM) showed the highest hCA I inhibitory activity and **6q** ($IC_{50} = 8.10$ μM) showed the highest hCA II inhibitory activity.

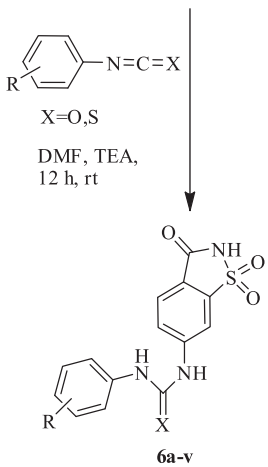
It was reported[7] that saccharin most likely coordinates in a deprotonated state through its nitrogen atom to the catalytically active zinc ion. Additionally, ureido-substituted benzenesulfonamide moieties were evidenced when the inhibitor was bound within the enzyme active site[10,11]. We believe that the synthesized saccharin urea/thiourea derivatives inhibited hCA I and II in the same way.

Structure–activity relationships

Generally, we have seen that all compounds (excluding **6c** and **6r**) have a higher inhibitory activity on hCA II than hCA I in the SARs study. When same substituents bonded to phenyl ring, most of the thiourea derivatives (**6l–v**) exhibited higher hCA I and hCA



	6a	6b	6c	6d	6e	6f	6g	6h
X	O	O	O	O	O	O	O	O
R	H	3-OCH_3	4-OCH_3	4-CH_3	3-Cl	4-Cl	3,4-di-Cl	3-NO_2
	6i	**6j**	**6k**	**6l**	**6m**	**6n**	**6o**	**6p**
X	O	O	O	S	S	S	S	S
R	4-NO_2	2-F	4-F	H	4-CH_3	4-OCH_3	2-F	3-F
	6q	**6r**	**6s**	**6t**	**6u**	**6v**		
X	S	S	S	S	S	S		
R	4-F	3-I	3-Cl	2,4-di-Cl	3,5-di-Cl	4-NO_2		



Scheme 1. Synthesis of 6-(phenylurenyl/thiourenyl) saccharin (**6a–v**) derivatives.

Table 1. Inhibitory effects of saccharin derivatives on hCA I and hCA II.

Compound	X	R	hCA I IC_{50} (μM)	hCA II IC_{50} (μM)	Compound	X	R	hCA I IC_{50} (μM)	hCA II IC_{50} (μM)
5	–	–	54.90	49.00	**6l**	S	H	46.74	40.24
6a	O	H	63.06	21.13	**6m**	S	4-CH_3	13.67	11.14
6b	O	3-OCH_3	41.65	6.54	**6n**	S	4-OCH_3	43.31	30.32
6c	O	4-OCH_3	34.55	37.66	**6o**	S	2-F	74.90	30.23
6d	O	4-CH_3	30.81	24.65	**6p**	S	3-F	46.70	14.55
6e	O	3-Cl	38.99	17.79	**6q**	S	4-F	55.87	8.10
6f	O	4-Cl	63.03	23.67	**6r**	S	3-I	20.21	30.70
6g	O	3,4-di-Cl	37.37	12.67	**6s**	S	3-Cl	33.41	17.19
6h	O	3-NO_2	37.47	32.74	**6t**	S	2,4-di-Cl	59.38	12.48
6i	O	4-NO_2	54.24	38.87	**6u**	S	3,5-di-Cl	26.10	11.04
6j	O	2-F	44.71	41.61	**6v**	S	4-NO_2	47.73	43.49
6k	O	4-F	42.64	17.14					

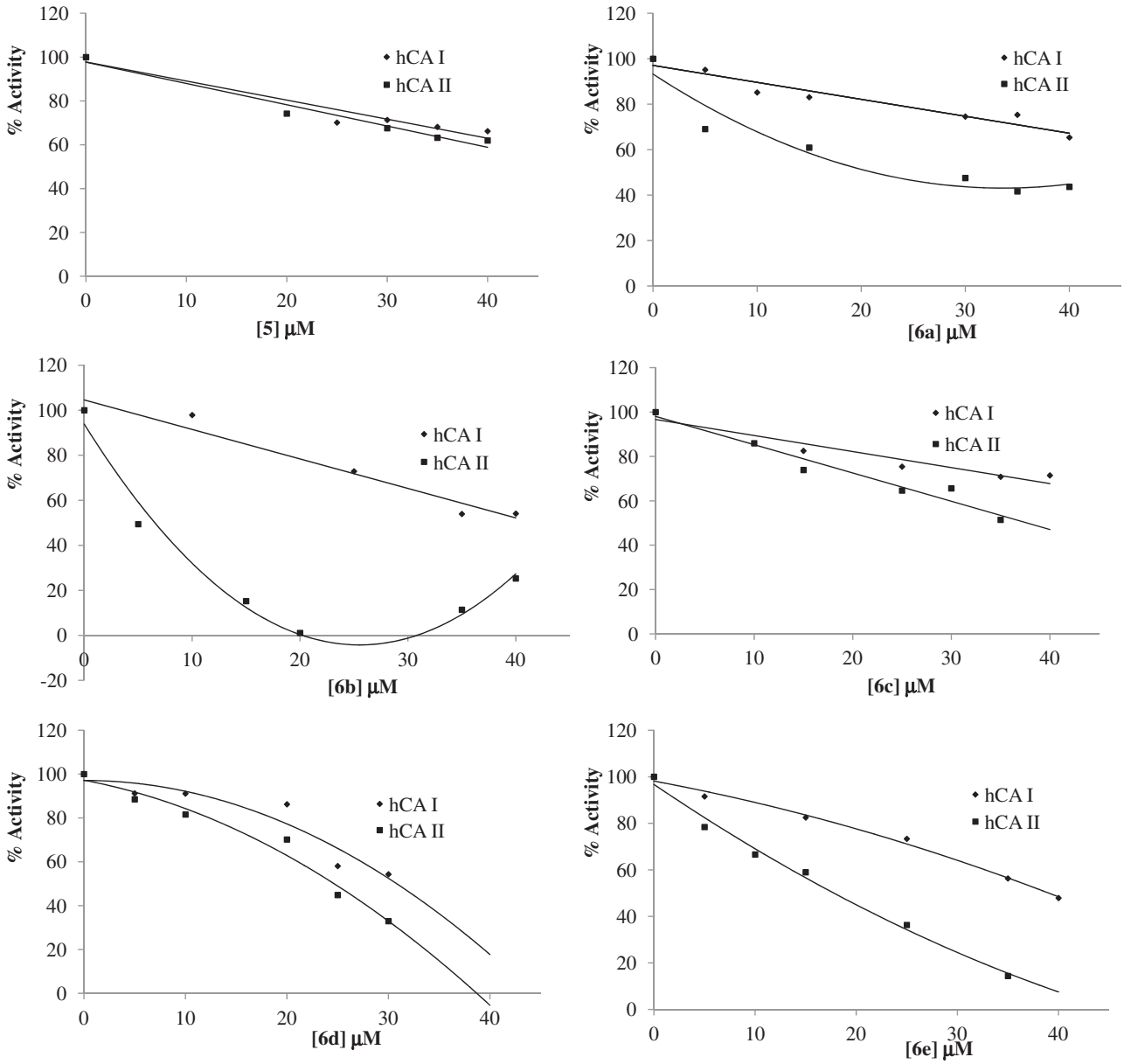


Figure 1. IC_{50} graphics of saccharin derivatives (5, 6a–v) on hCA I and hCA II.

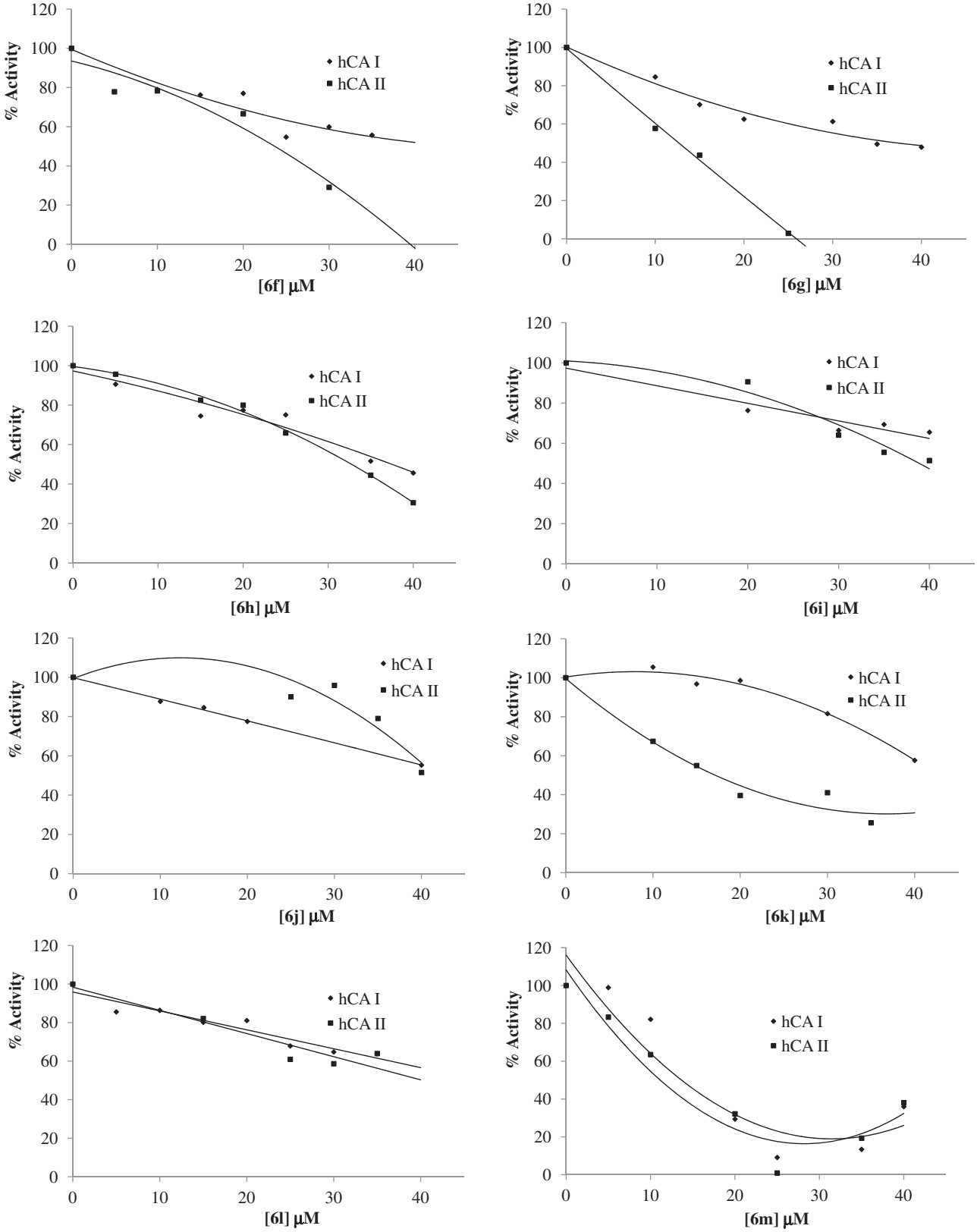


Figure 1. Continued.

II inhibitory activity than urea derivatives (**6a–k**). Additionally, the following results were obtained.

(a) For urea derivatives:

(i) Although electron-withdrawing groups (nitro and halogens) bonded to *meta* position of the phenyl ring (**6e**, **6g** and **6h**) increased the inhibitory activity on hCA I, electron-donating groups (methoxy) bonded to *meta* position of phenyl ring (**6b**) had the highest hCA II inhibitory activity ($IC_{50} = 6.54\,\mu M$).

(ii) Electron-donating groups (methoxy, methyl) bonded to *para* position of the phenyl ring (**6c** and **6d**) inhibited hCA I activity more than halogens and electron-withdrawing groups bonded. On the other hand, halogen groups bonded to the *para* position of

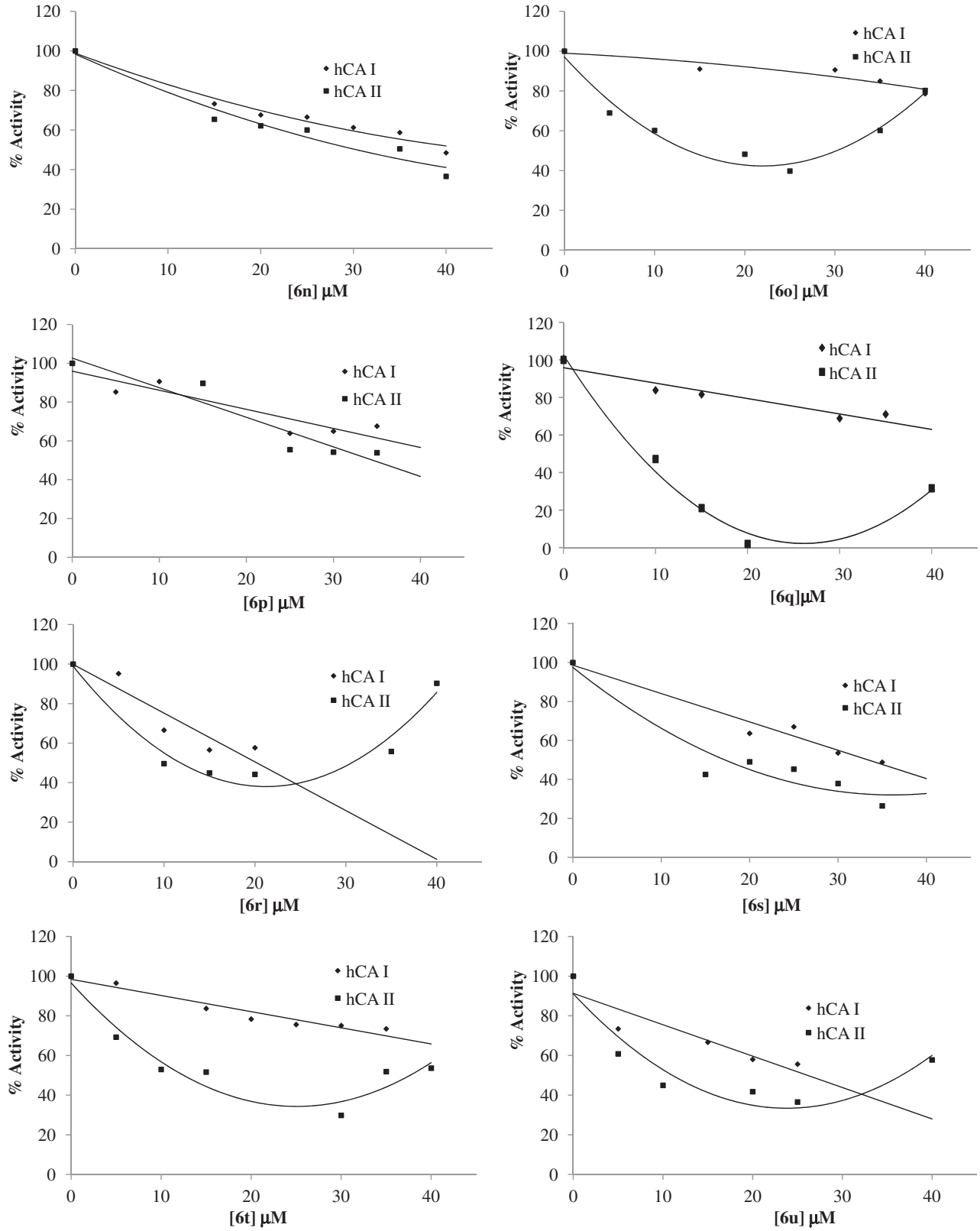


Figure 1. Continued.

the phenyl ring (**6f**, **6g** and **6k**) increased the inhibitory activity on hCA II.

(b) For thiourea derivatives:
  (i) Electron-donating groups bonded to the *para* position of the phenyl ring (**6m** and **6n**) increased the hCA I inhibitory activity.
  (ii) Moving-F group on the phenyl ring from *ortho* (**6o**, $IC_{50} = 30.23\,\mu M$) to *meta* (**6p**, $IC_{50} = 14.55\,\mu M$) and *para* (**6q**, $IC_{50} = 8.10\,\mu M$) positions led to major enhancement of hCA II inhibitory activity.
  (iii) In same moving for hCA I activity, **6p** ($IC_{50} = 46.70\,\mu M$) was more inhibited than **6o** ($IC_{50} = 74.90\,\mu M$) and **6q** ($IC_{50} = 55.87\,\mu M$).
  (iv) Halogen series on the *meta* position of the phenyl ring showed a linear relationship for higher hCA I inhibitory activity with increasing size and

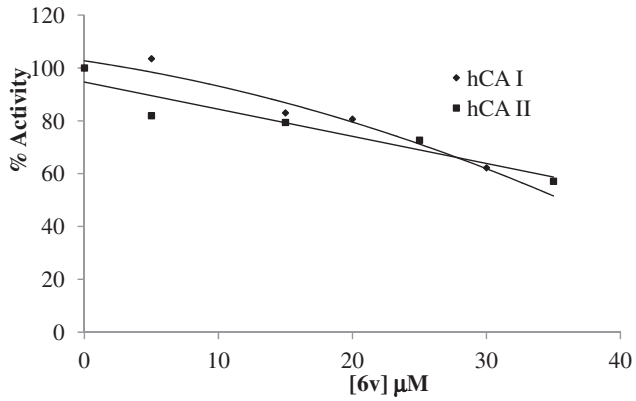


Figure 1. Continued.

polarizability (for size and polarizability, I > Cl > F, for hCA I inhibitory activity, **6r** ($IC_{50} = 20.21\ \mu M$) > **6s** ($IC_{50} = 33.41\ \mu M$) > **6p** ($IC_{50} = 46.70\ \mu M$). Interestingly, this series showed an inverse relationship for hCA II inhibitory activity with increasing size and polarizability (for hCA II inhibitory activity, **6r** ($IC_{50} = 30.70\ \mu M$) < **6s** ($IC_{50} = 17.19\ \mu M$) < **6p** ($IC_{50} = 14.55\ \mu M$).

Conclusions

In conclusion, we evaluated 6-(phenylurenyl/thiourenyl) saccharin derivatives (**6a–v**) effects on hCA I and hCA II purified from human erythrocytes and SARs were examined. All compounds inhibited both hCA I and hCA II enzyme activities. Most of the compounds had higher hCA II inhibitory activity than hCA I activity. Most of the thiourea derivatives (**6l–v**) exhibited higher hCA I and hCA II inhibitory activities than urea derivatives (**6a–k**). The present study revealed that activity could also be influenced by the type and position of the substituent on the phenyl ring. Among all compounds, **6m** showed the highest hCA I inhibitory activity and **6b** showed the highest hCA II inhibitory activity.

In summary, enzyme inhibition is the most important issue for drug design and biochemical applications[15–27]. Therefore, our results suggested that saccharin 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 conflicts of interest.

This work was supported by Sakarya University Scientific Research Project (Project No. 2011–50-02-020).

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