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# Carbohydrazones as new class of carbonic anhydrase inhibitors: Synthesis, kinetics, and ligand docking studies

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ABSTRACT

Discovery and development of carbonic anhydrase inhibitors is crucial for their clinical use as antiepileptic, diuretic and antiglaucoma agents. Keeping this in mind, we have synthesized carbohydrazones **1**–**27** and evaluated them for their *in vitro* carbonic anhydrase inhibitory potential. Out of twenty-seven compounds, compounds **1** ($IC_{50}$ = 1.33 ± 0.01 μM), **2** ($IC_{50}$ = 1.85 ± 0.24 μM), **3** ($IC_{50}$ = 1.37 ± 0.06 μM), and **9** ($IC_{50}$ = 1.46 ± 0.12 μM) have showed carbonic anhydrase inhibition better than the standard drug zonisamide ($IC_{50}$ = 1.86 ± 0.03 μM). Moreover, compounds **4** ($IC_{50}$ = 2.32 ± 0.04 μM), **5** ($IC_{50}$ = 3.96 ± 0.35 μM), **7** ($IC_{50}$ = 2.33 ± 0.02 μM), and **8** ($IC_{50}$ = 2.67 ± 0.01 μM) showed good inhibitory activity. Cheminformatic analysis has shown that compounds **1** and **2** possess lead-like properties. In addition, kinetic and molecular docking studies were also performed to investigate the binding interaction between carbohydrazones and carbonic anhydrase enzyme. This study has identified a novel and potent class of carbonic anhydrase inhibitors with the potential to be investigated further.



## 1. Introduction

During the last decade, carbohydrazones or *bis*-Schiff bases of carbohydrazide have received major attention of medicinal chemists due to the presence of multiple potential donor sites to form mononuclear, dinuclear and even tetranuclear molecular square grid complexes [1–4], thereby providing unique supramolecular assemblies. In last few years, several reports describing the transition metal complexes of these ligands have been appeared in literature [5–11]. Moreover, these compounds also exhibit antimicrobial activity towards bacteria and fungi [12,13].

Carbohydrazide has structural sequence of urea and semicarbazide [14] therefore, can react with carbonyl compounds from both ends to afford *bis*-Schiff bases. Hydrazones are not only used as biologically active compounds but also as analytical reagents. Hydrazones play vital role in the treatment of diseases such as leishmania, diabetes, tumor, tuberculosis, leprosy and mental disorder [15–18]. Hydrazones also find applications in detection, determination and isolation of compounds having carbonyl moiety. More recently, they have been extensively used in the detection of several metals [19].

Carbonic anhydrases (EC 4.2.1.1) are metalloproteins, found in mammals. They are divided into four major subgroups, further comprised of several isoforms. Carbonic anhydrase (CA-II) contains a tightly bound $Zn^{2+}$ at the active site. The $Zn^{2+}$ cation is bound with one water and three histidine molecules. The function of $Zn^{2+}$ cation, a strong Lewis acid, is to bind and activate substrate water molecule to catalyze the reversible hydration reaction of carbon dioxide into bicarbonate as shown in Fig. 1. In the absence of CA enzymes, hydration of carbon dioxide does not proceed at a considerable rate under physiological conditions [20]. CA enzymes play a pivotal role in diverse processes, such as physiological pH control, gas balance, calcification, respiration and secretion of electrolytes. Carbonic anhydrase inhibitors are known to serve as antiepileptic, diuretic and antiglaucoma agents [21–25].

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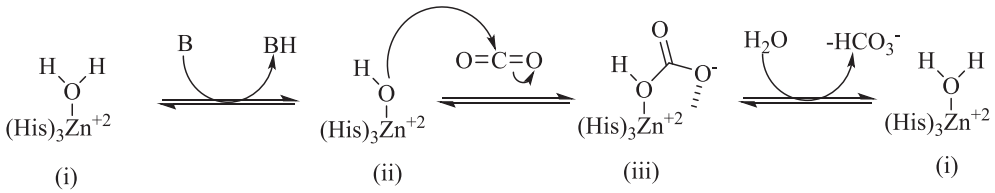


**Fig. 1.** Mechanism of action of carbonic anhydrase.

Carbonic anhydrase inhibitors have been used in the treatment of glaucoma for past three decades. CA inhibition has a crucial role in cancer treatment through reducing the provision of bicarbonate for the synthesis of nucleotides and other cell components, such as membrane lipids [26]. Currently, carbonic anhydrase inhibitors are administered systemically, and include acetazolamide, dichlorophenamide, ethoxzolamide and methazolamide [27–29]. A number of carbonic anhydrase inhibitors has been reported to lower the intraocular pressure when instilled topically in animals. Intraocular pressure is decreased by reduction in humor formation, stemming from the inhibition of carbonic anhydrase present in the ciliary epithelium [30,31].

To the best of our knowledge, this is the first report describing the carbonic anhydrase inhibitiory activity of carbohydrazones. To date, all known inhibitors of carbonic anhydrase, *i.e.*; acetazolamide, dichlorophenamide, ethoxzolamide, and methazolamide, belong to sulphonamide class of compounds [32]. As several side effects are associated with sulphonamides along with their involvement in the inhibition of other enzymes for instance, Celecoxib®, a non-selective carbonic anhydrase inhibitor, involved in the inhibition of COX-1, COX-2, and phosphodiesterase-5 enzyme. Therefore, there is an urgent need to explore other classes of compounds having selective carbonic anhydrase inhibitory activity without cytotoxicity. Keeping this in mind, we have synthesized several classes of small molecules which may act as selective carbonic anhydrase inhibitors [11,17,18,33]. In current study, we choose carbohydrazone class of compounds particularly based on mechanism of action of carbonic anhydrase depicting in Fig. 1.

Our assumption was that a compound with sterically unhindered carbonyl moiety may act as carbonic anhydrase inhibitor. To test our hypothesis, we synthesized a variety of carbohydrazones (**1**–**27**) and screened them for carbonic anhydrase inhibitory activity. A proposed mechanism of action of carbohydrazones in carbonic anhydrase inhibition is shown in Fig. 2.

## 2. Results and discussion

### 2.1. Chemistry

Synthesis of lead molecules against well-defined biological targets is an ongoing research of our group. In recent years, we have reported Schiff bases exhibiting diverse biological activities [11,17,18,33]. In the light of these findings, we designed our current project. In this report, *in vitro* inhibition of bovine carbonic anhydrase II by carbohydrazone derivatives of the general structure, shown in Table 1, is reported.

Carbohydrazones **1**–**27** were synthesized by reacting commercially available carbohydrazide and a range of aromatic aldehydes (Scheme 1). In a typical reaction, few drops of acetic acid were added to a stirred mixture of substituted aromatic aldehyde (4.0 mmol) and carbohydrazide (2.0 mmol) in anhydrous ethanol. The reaction mixture was refluxed and the reaction progress was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature; the crude product obtained was filtered and washed with hexanes. After crystallization from ethanol, the pure carbohydrazones **1**–**27** were obtained in excellent yields. The structures of all the synthetic compounds were deduced by using spectroscopic techniques ($^1$H NMR, EI-MS, HREI-MS, TOF-MS, and HRTOF-MS). Elemental analyses were found to be in agreement with the calculated values. In addition, $^{13}$C NMR and IR spectroscopy was performed on new compounds **11**, **17**, **24**, and **27**.

### 2.2. General structure elucidation of compounds by spectroscopic techniques

The structures of all synthetic compounds were deduced by spectroscopic techniques. The $^1$H NMR spectrum of representative compound **6** was recorded in deuterated DMSO. Since, there is symmetry in the molecule so only half of the molecule appeared in $^1$H and $^{13}$C NMR spectra. A singlet at $\delta$ 10.78 showed the presence of benzilidene proton while a broad singlet at $\delta$ 8.16 indicated the presence of an amide proton. The phenyl ring C-2/C-6 protons coupled with C-3/C-5 protons and appeared as doublet at $\delta$ 7.78 ($J_{2,3} = J_{6,5} = 8.4$ Hz). Likewise, C-3/C-5 protons appeared as doublet at $\delta$ 7.50 ($J_{3,2} = J_{5,6} = 8.4$ Hz) (Fig. 3).

The $^{13}$C NMR spectrum of compound **6** was recorded in deuterated DMSO. Signals appeared at $\delta$ 128.73, 128.40, and 141.8 represented C-2/C-6, C-3/C-5 and methylene carbon (=CH), respectively. Quaternary C-1, C-4 and C=O appeared at $\delta$ 133.6, 133.8 and 151.9, respectively (Fig. 4).

Geometrical isomerism was confirmed by NOE analysis in which signal enhancement was typically observed for amidic protons by irradiating benzilidine protons, likewise signal enhancement was also observed for benzilidine protons, on irradiating the amidic protons indicating that they are located on the same side.

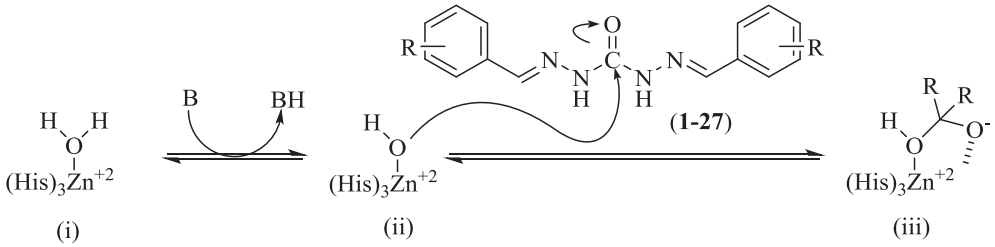


**Fig. 2.** A proposed mechanism of inhibition of carbonic anhydrase with carbohydrazone.