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SHORT COMMUNICATION

The inhibitory effects of phenolic Mannich bases on carbonic anhydrase I and II isoenzymes

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Abstract

Phenolic mono Mannich bases [2-[4-hydroxy-3-(aminomethyl)benzylidene]-2,3-dihydro-1H-inden-1-one (**8–15**)] and bis Mannich bases [2-[4-hydroxy-3,5-bis(aminomethyl)benzylidene]-2, 3-dihydro-1H-inden-1-one (**2–7**)] were synthesized starting from 2-(4-hydroxybenzylidene)-2, 3-dihydro-inden-1-one (**1**). This study was designed in order to investigate the carbonic anhydrase (CA, EC 4.2.1.1) inhibitory properties of a library of compounds incorporating the phenol functional group. All prepared compounds showed a low inhibition percentages on both human (h) isoforms hCA I and hCA II compared to the reference sulfonamide acetazolamide. Mannich bases **2–15** had lower inhibition percentages than the compound **1** on hCA I and hCA II, except compound **14**, which is a Mannich base derivative of dipropylamine, which had a similar inhibitory power as compound **1** on hCA II. All compounds synthesized **1–15** were 1.3–1.9 times more effective on hCA II comparing with the effectiveness of the compounds on hCA I.

Keywords

Carbonic anhydrase, indanone, Mannich bases, phenol

History

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Introduction

Carbonic anhydrases (CA, EC 4.2.1.1) are zinc metalloproteins, which catalyze a very simple reaction: the carbon dioxide (CO₂) hydration reaction to bicarbonate and proton. CA isoforms are found in a variety of tissues where they participate in several important biological processes such as acid–base balance, respiration, CO₂ and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis, and electrolyte secretion. Many CA isozymes involved in these processes

are important therapeutic targets with the potential to be inhibited/activated for the treatment of a range of disorders such as edema, glaucoma, obesity, cancer, epilepsy, and osteoporosis¹.

Some phenol-bearing compounds are reported with their CA inhibitory activities². The simple phenol molecule was reported to act as an inhibitor of the zinc enzyme CA in a different mechanism of action³. Indeed, sulfonamides coordinate to the metal ion from the enzyme active site; phenols and derivatives anchor to the water molecule/hydroxide ion coordinated to the metal ion^{4,5}.

The side effects of many compounds belonging to the first-/second generation of carbonic anhydrase inhibitors (CAIs) of types 1–15 (among which metabolic acidosis, kidney stones, bone loss, and so on) are due to the potent inhibition of all mammalian CA isoforms, and not only of the target one. The main scope of the drug

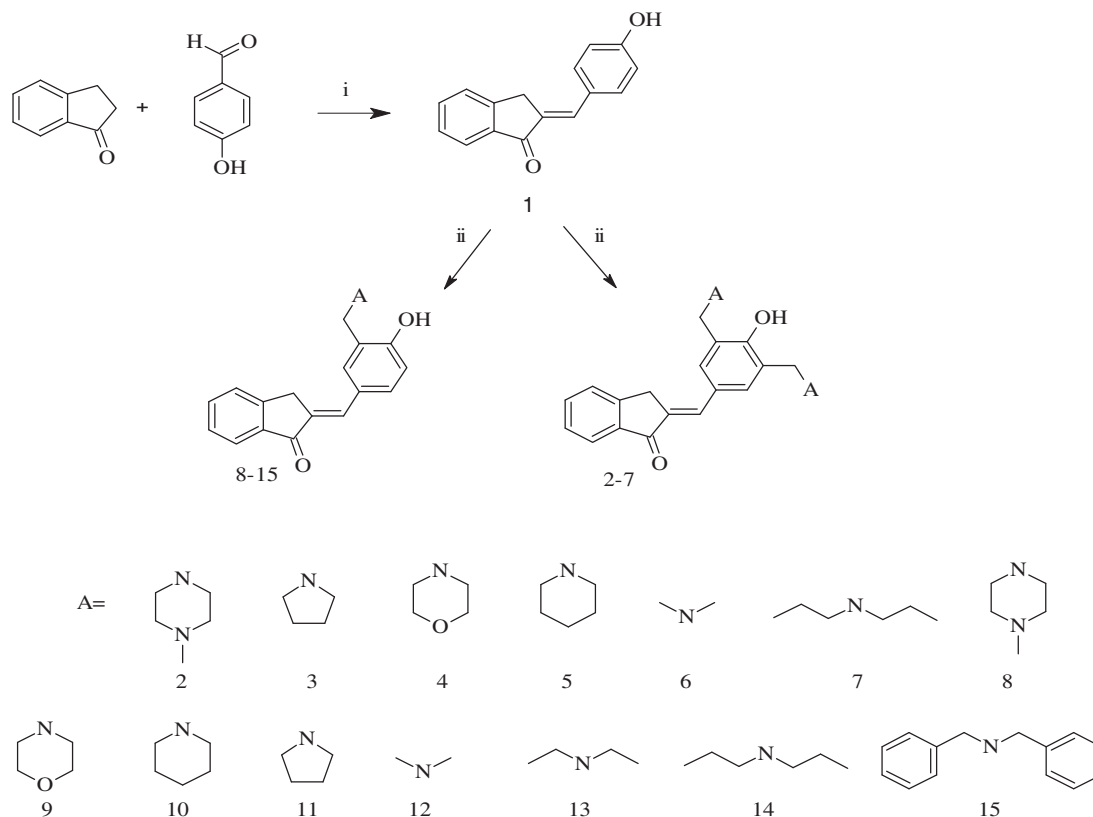
is to obtain isoform-selective CAIs for the various isoforms involved specifically in different pathologies^{5–8}.

Chalcones have α - β -unsaturated ketone moiety in its structure and they are common structure in natural products such as flavonoids⁹. Chalcones and its analogs are excellent scaffolds for synthetic manipulations and also possess multiple biological and medicinal properties. Indanone-derived compounds were also reported with several biological activities such as cytotoxic, antioxidant, anticholinergic, antifungal, and anticancer activities¹⁰.

Mannich bases are synthesized by Mannich reaction. It is an aminomethylation process of a compound or a drug. They are an important group of compounds in medicinal chemistry. Aminomethylation of drugs could be used to improve the delivery of a drug into the human body. Aminomethylation may increase the hydrophilic properties of drugs through the introduction of a polar function into the chemical structure. The aminomethylated drugs could act as prodrugs releasing the active substance under controlled hydrolytic conditions via deamination. There are several type of Mannich bases such as carbon Mannich bases and nitrogen Mannich bases¹¹. Phenolic Mannich bases are a group of carbon Mannich bases. It was reported CAs inhibitory^{12–14}, cytotoxic^{15–18}, anticonvulsant^{19–21}, antiinflammatory²², diuretic²³, and antifungal²⁴ activities of Mannich bases.

In this study, it was aimed to investigate the CA inhibitory activities of new phenolic chalcone analog compounds having indanone skeleton to make a contribution to CA research library and give some insight to the researchers who are interested in CA research. For this aim, it was planned to use some phenolic mono Mannich bases [(2-[4-hydroxy-3-(aminomethyl)benzylidene]-2,3-dihydro-1H-inden-1-one (**8–15**, Scheme 1)] and phenolic bis Mannich bases [(2-[4-hydroxy-3,5-bis(aminomethyl)benzylidene]-2,3-dihydro-1H-inden-1-one (**2–7**, Scheme 1)] synthesized

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Scheme 1. General structures of the compounds synthesized. i: Ethanol, aq. NaOH 10% ii: Paraformaldehyde, amine (A), acetonitrile, 200W, 13.8 barr, 120°C Amine used: Corresponding amines of A.

according to our previous studies^{17,25} starting from 2-(4-hydroxybenzylidene)-2,3-dihydro-inden-1-one (**1**, Scheme 1).

Material and methods

CA enzyme assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO₂ hydration activity²⁶. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na₂SO₄ (for maintaining constant ionic strength), following the initial rates of the CA-catalyzed CO₂ hydration reaction for a period of 10–100s. The CO₂ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition percentage was obtained by using PRISM 3, as reported earlier²⁷, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier²⁸. The cell pellets were lysed, and hCA II and hCA I were purified through affinity chromatography using pAMBS resin.

Results and discussion

The hCA I and hCA II inhibition percentages of the compounds **1–15** are shown in Table 1 and also in Figure 1. According to

Table 1. Inhibition percentages of compound **1–15** on hCA I and hCA II isoenzymes at 1 μM inhibitor concentration in the assay system.

Compound No.	% Inhibition		% Inhibition		hCA II/hCA I
	hCA I	A	hCA II	B	
1	35	–	45	–	1.3
2	25	1.4	38	1.2	1.5
3	26	1.3	39	1.2	1.5
4	31	1.1	42	1.1	1.4
5	26	1.3	41	1.1	1.6
6	26	1.3	36	1.3	1.4
7	28	1.3	37	1.2	1.3
8	23	1.5	41	1.1	1.8
9	23	1.5	39	1.2	1.7
10	31	1.1	42	1.1	1.4
11	24	1.5	41	1.1	1.7
12	26	1.3	43	1.0	1.7
13	29	1.2	43	1.0	1.5
14	25	1.4	45	1.0	1.8
15	23	1.5	44	1.0	1.9
AAZ	79	–	95	–	–

AAZ: Acetazolamide used as a positive control.

Inhibition concentration was 10^{–6} M for all compounds.

“A” values were calculated by dividing the inhibition percentage value of the compound **1** against hCA I to inhibition percentage value of the Mannich bases against hCA I.

“B” values were calculated by dividing the inhibition percentage value of the compound **1** against hCA II to inhibition percentage value of the Mannich bases against hCA II.

Table 1, Mannich bases **2–15** had lower inhibition percentage on hCA I than the compound **1** from which Mannich bases were derived from. Only the compound **14** had equal inhibition percentage with the compound **1** on hCA II. All compounds had lower inhibition percentages than reference drug

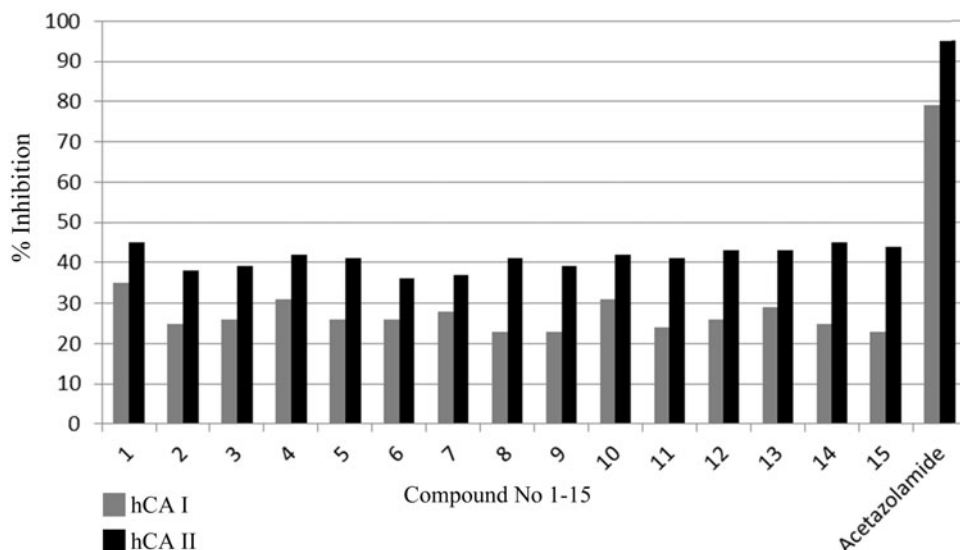


Figure 1. CA inhibition percentages of the compounds on graphical model.

acetazolamide (AAZ) on both hCA I and hCA II. According to the results in Table 1, bis Mannich bases compound **2** (with *N*-methyl piperazine), compound **3** (with pyrrolidine), compound **4** (with morpholine), and compound **7** (with dipropylamine) did more inhibition on hCA I than their corresponding mono Mannich bases.

The inhibition potency of the bis Mannich bases at issue in terms of times for the compound **2**, **3**, and **7** were 1.1 times while it was 1.3 times for the compound **4** comparing to their corresponding mono Mannich bases. On the other hand, mono Mannich base **10** (with piperidine) had shown 1.2 times more potent hCA I inhibition than its corresponding bis Mannich base analog, the compound **5**.

When inhibitory activities of the compounds on hCA II were considered, mono Mannich bases were more effective than bis Mannich bases on the contrary to hCA I's. It can be easily noticed from Table 1 that mono Mannich bases **8** (with *N*-methyl piperazine), **11** (with pyrrolidine), **12** (with dimethylamine), and **14** (with dipropylamine) were more effective than their corresponding bis Mannich bases on hCA II. The potency ratios were 1.1 times stronger for the compounds **8** and **11**, and 1.2 times stronger for the compounds **12** and **14** comparing to their corresponding bis Mannich bases.

The compound **1** was more effective on hCA I (1.1–1.5 times) and hCA II (1.1–1.3), compared with the Mannich bases **2–15**. The Mannich bases reported here show poor inhibitory activity against these two CA isoforms. This is probably due to the fact that in the *ortho* position/s to the OH moiety there are one or two rather bulky functionalities which may interfere with the binding of the compound to the enzyme active site. In addition, the compounds **1–15** were 1.3–1.9 times more effective on hCA II than on hCA I without exception according to results in Table 1 and Figure 1.

Conclusion

All compounds had lower inhibition percentages on both hCA I and hCA II than reference compound AAZ. Mannich bases **2–15** had lower inhibition percentages than the compound **1** on both hCA I and hCA II. Exception was compound **14** with dipropylamine, which has equal inhibition percentage with the compound **1** on hCA II. All compounds synthesized (**1–15**) were 1.3–1.9 times more effective on hCA II compared with the

inhibition on hCA I. The Mannich bases reported here show more poor inhibitory activity against these two CA isoforms than starting compound which is chalcone analog. This is probably due to the fact that in the *ortho* position to the OH moiety there are one or two rather bulky functionalities which may interfere with the binding of the compound to the enzyme active site. By this study, CA inhibitory activities of new phenolic compounds were reported to give some insight to the scientists focusing on CA research.

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Declaration of interest

The authors report no conflict of interest and are responsible for the contents and writing of the paper.

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