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Short communication

Synthesis of *N*′-phenyl-*N*-hydroxyureas and investigation of their inhibitory activities on human carbonic anhydrases

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ABSTRACT

A series of *N*′-phenyl-*N*-hydroxyureas has been prepared by reacting hydroxylamine with aromatic isocyanates. These compounds were investigated as inhibitors of human carbonic anhydrases (hCAs, EC 4.2.1.1), considering four physiologically relevant isoforms, the cytosolic isoforms hCA I and II, and tumor associated, transmembrane isoforms hCA IX and XII. The new compounds reported here did not inhibit the widespread cytosolic isoforms hCA I and II, but they inhibited the tumor associated isoforms with interesting potencies. The most effective inhibitors showed K_Is ranging between 72.8 and 78.9 nM against hCA IX and between 6.9 and 7.2 against hCA XII, making them of interest as candidates for antitumor studies.

1. Introduction

The hydration reaction of carbon dioxide in cells and organisms is reversibly catalyzed by the carbonic anhydrases (CAs, EC 4.2.1.1) enzymes [1,2]. This reaction also occurs spontaneously, however when only the uncatalyzed process is considered, it does not meet the physiological requirements for converting the high amounts of metabolically generated CO_2 into soluble products, bicarbonate and protons [3–5]. To date seven genetically diverse CA families have been characterized (α-, β-, γ-, δ-, ζ-, η- and θ-CAs) and they are all not related each other [1–8]. The fifteen α-CA isoforms identified in humans (h) differ for their kinetic properties, sub-cellular localization as well as tissue distributions [1–2,6–12]. From the physiological viewpoint, in higher vertebrates, such as the humans, they are involved in vital processes, which among others include pH homeostasis, respiration, transportation of CO_2/bicarbonate, etc. In addition, CAs are also involved in fundamental biosynthetic transformations (gluconeogenesis, ureagenesis, lipogenesis, etc.) in which CO_2 or bicarbonate are substrates of various enzymes [1–2,9–14]. Thus, it is not surprising that when abnormal levels of CAs are detected, a pathological process is taking place. In this context the use of CA inhibitors (CAIs) is a common pharmacological approach to treat various disease such as hypertension, elevated intraocular pressure associated diseases (glaucoma), epilepsy, obesity and altitude sickness [9–27]. The most common CAIs reported to date possess the primary sulfonamide ($-SO_2NH_2$) or the bioisosteric sulfamate ($-OSO_2NH_2$) and sulfamide ($-NHSO_2NH_2$) pharmacophores, which tightly bind the catalytic zinc ion located at the bottom of the enzymatic cleft [13–32]. In the last two decades new types of CAIs have been identified, such as the dithiocarbamates, the xanthates and the monothiocarbamates, which coordinate the zinc ion in a similar manner as the sulfonamides [33–36]. The phenols, the carboxylic acids, the polyamines, and the sulfonic acids obtained from *in situ* hydrolyzed sulfocoumarins, anchor to the zinc-bound water molecule, inhibiting the enzyme by a diverse mechanism compared to the zinc binders [37–47]. Finally the coumarins and thiocoumarins were reported to inhibit the CAs by means of occlusion of the active site entrance [48,49]. Recently we reported compounds of the 2-(benzylsulfonyl)-benzoic acid type exerting their inhibitory activity while located outside of the active site cavity, in an adjacent hydrophobic pocket nearby the entrance of the active site [50]. On the pursuit of versatile chemical moieties to be further investigated for CA inhibition, we turned our attention to the *N*-hydroxy-ureas or hydroxamic acids of the type **A-C** below reported (Fig. 1) [51,52].

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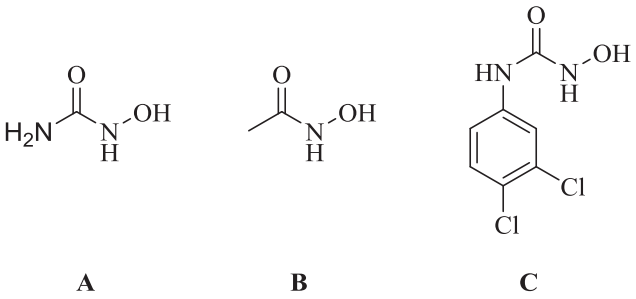


Fig. 1. Previously reported the *N*-hydroxy-ureas (**A**) or hydroxamic acids (**B**, **C**) CAIs [51,52].

As the introduction of the phenyl pendant into the hydroxamic acid moiety was reported to lead to a significant improvement of both the inhibitory profiles and potencies against various hCAs compared to the lead **A** [52], we turned our attention to develop a series of *N*′-phenyl-*N*-hydroxyureas, because only one such compound was investigated, derivative **C** [52]. All the compounds obtained here were explored for their in vitro inhibition against four physiological relevant CA isoforms (hCA I, II, IX and IX).

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **1**–**22** reported here was carried out according to literature reports [53]. Hydroxyl amine hydrochloride was reacted with a series of aromatic and commercially available isocyanates in the presence of triethylamine, thus leading to the corresponding *N*′-phenyl-*N*-hydroxyureas with simple substitution patterns, including methyl/halogens/nitro/methoxy/ethoxy/ethyl benzoate/phenoxy/5-indanyl and naphthalen-1-yl. Compounds **1**–**5**, **7**–**10**, **13**, **17** and **22** were previously reported in literature (Scheme 1) [53–58].

2.2. Carbonic anhydrase inhibition

The series here reported (compounds **1**–**22**) was investigated for inhibitory effects against four physiological relevant isoforms, i.e., hCA I, II, IX and XII, by means of a stopped flow CO_2 hydrase assay [59].

I Compounds **1**–**22** were ineffective inhibitors of the cytosolic, abundant isoforms hCA I and II (K_Is > 10000 nM) (See Table 1).

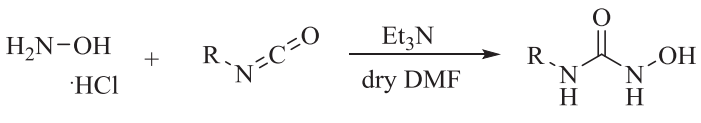
Table 1
Inhibition data of human CA isoforms hCA I, II, IX and XII with compounds **1**–**22** in comparison with the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay [59].

	K_I (nM)*			
Cmp	hCA I	hCA II	hCA IX	hCA XII
1	>10,000	>10,000	>10,000	27.4
2	>10,000	>10,000	>10,000	253.2
3	>10,000	>10,000	>10,000	>10,000
4	>10,000	>10,000	8237.3	491.2
5	>10,000	>10,000	>10,000	>10,000
6	>10,000	>10,000	>10,000	808.8
7	>10,000	>10,000	>10,000	>10000
8	>10,000	>10,000	7781.7	43.6
9	>10,000	>10,000	>10,000	529.2
10	>10,000	>10,000	>10,000	768.0
11	>10,000	>10,000	>10,000	858.2
12	>10,000	>10,000	>10,000	746.6
13	>10,000	>10,000	78.9	7.2
14	>10,000	>10,000	679.1	27.9
15	>10,000	>10,000	253.5	>10,000
16	>10,000	>10,000	>10,000	>10,000
17	>10,000	>10,000	268.9	51.3
18	>10,000	>10,000	>10,000	>10,000
19	>10,000	>10,000	130.0	42.1
20	>10,000	>10,000	>10,000	377.6
21	>10,000	>10,000	72.8	6.9
22	>10,000	>10,000	86.4	33.6
AAZ	250	12.1	25.8	5.7

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ±5–10% of the reported values).

II The compounds **1**–**22** showed a variety of inhibitory potencies towards the tumor associated, transmembrane isoform hCA IX. For instance, this isoform was effectively inhibited by derivatives **13, 21** and **22** with K_Is spanning between 72.8 and 86.4 nM. Alternatively, **15**, **17** and **19** were medium potency inhibitors of the IX isoform with K_Is in the range of 130–268.9 nM, followed by compounds **4**, **8**, **14** (K_Is comprised between 0.68 and 8.2 μM). Finally the remaining compounds in the series (**1–3, 5–7, 9–12, 16, 18, 20**) were ineffective inhibitors with K_Is > 10 μM.

III The second tumor associated and transmembrane isoform hCA XII, was inhibited by most of the compound in this series with K_Is spanning between 6.9 and 858.2 nM. The most effective inhibitors were **13** and **21** (K_Is of 7.2 and 6.9 nM respectively). Compounds **1**, **8**, **17**, **19**, and **22** were also effective inhibitors (K_Is of 27.4–51.3 nM), whereas the **2**, **4**, **6**, **9**–**12** and **20** derivatives resulted to be weak inhibitors of this isoform, with K_Is between 253.2 and 858.2 nM. The remaning compound **3, 5, 7, 15, 16** and **18** were ineffective against hCA XII.



HA **1a-v** **1-22**

1: R = Ph
2: R = 4-$CH_3C_6H_4$
3: R = 2-$CH_3C_6H_4$
4: R = 4-ClC_6H_4
5: R = 3-ClC_6H_4
6: R = 2-ClC_6H_4
7: R = 4-FC_6H_4
8: R = 4-$O_2NC_6H_4$
9: R = 2-$O_2NC_6H_4$
10: R = 2-$MeOC_6H_4$
11: R = 2-$EtOC_6H_4$
12: R = 4-$PhOC_6H_4$
13: R = 3-$MeSC_6H_4$
14: R = 3-$EtOOCC_6H_4$
15: R = 2,5-$Me_2C_6H_3$
16: R = 3,5-$Me_2C_6H_3$
17: R = 4-$CF_3C_6H_4$
18: R = 4-F- 3-MeC_6H_3
19: R = 4-CF_3- 2-ClC_6H_3
20: R = 2,3,4,5,6-F_5
21: R = indan-5-yl
22: R = naphthalen-1-yl

Scheme 1. Synthesis of compounds **1**–**22**.