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Carbonic anhydrase I and II activation with mono- and dihalogenated histamine derivatives

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ABSTRACT

Mono- and dihalogenated histamine derivatives incorporating fluorine, chlorine and bromine have been prepared together with the corresponding boc-protected compounds at the aminoethyl group. They have been investigated as activators of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). The cytosolic human (h) isoforms hCA I and II were moderately activated by the boc-protected halogenated histamines and very effectively activated by the deprotected ones. Low nanomolar and subnanomolar hCA I and II activators have been detected for the first time, starting from histamine as lead which has an affinity of 2 μM against isoform I and of 125 μM against hCA II.

Activation of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) remains a rather neglected research field, in contrast to CA inhibition, which is widely investigated in the search of antitumor, antiglaucoma, diuretic, anticonvulsant and antiobesity therapies.[1–6] All 16 CA mammalian isoforms have been investigated for their activation with several classes of activators, most of which belong to the amine, amino acid and oligopeptide chemotypes.[7–10] The CA activation mechanism is thoroughly understood through the extensive X-ray crystallographic and kinetic work from several groups.[7,11–14] The rate-determining step for the CO_2 hydration reaction catalyzed by CAs is the proton transfer reaction from the water bound to the Zn(II) ion to the reaction medium, with generation of the zinc hydroxide species of the enzyme.[7,11–14]

This step is achieved in many α-CA isoforms through the participation of the active site residue His64, which being highly flexible acts as a proton shuttle between the active site and the reaction medium.[7,11–14] Compounds able to participate in such proton transfer reactions, of the amine, amino acid and oligopeptide type have been shown to act as efficient CA activators (CAAs).[1,7] It should be mentioned that the first X-ray crystal structure of an activator complexed to CA was the adduct of hCA II with histamine.[11] In this and subsequent work it has been shown that the activator binding site is at the entrance of the enzyme cavity, far away from the catalytic metal ion (Fig. 1). In such a position, the activators are able to participate in supplementary proton release pathways (to the one achieved through His64), with enhanced generation of the nucleophilic zinc-hydroxide species of the enzyme, leading thus to more efficient catalysis.[11] Many other adducts of activators complexed to various CA isoforms have been reported in the last period, such as, for example, the human (h) hCA II adducts with L-/D-Phe, L-/D-His, D-Trp, etc.[7,12–14] Furthermore, CAs possessing a lower catalytic activity (e.g., CA III, XIII, etc.) were also shown to be highly activatable with many classes of such activators, which provide alternative pathways to the natural shuttle residue for the proton release from the active site.[15,16] It has been proposed that CAAs may be effective for enhancing cognition in neurodegenerative diseases (Alzheimer's disease) or due to aging, since the level of brain CA isoforms is diminished in such conditions.[17]

As mentioned above, several X-ray crystal structures are available for adducts of the physiologically dominant isoforms hCA II with amine and amino acid CAAs. As shown in Figure 1, most of them bind in the same region of the active site, at its entrance, parallel to the natural proton shuttle residue, His64. Indeed, histamine, L-/D-His and L-/D-Phe bind in this way, in what we have denominated the activator binding site A.[7–11] Only D-Trp binds differently, towards the more external part of the active site (and is

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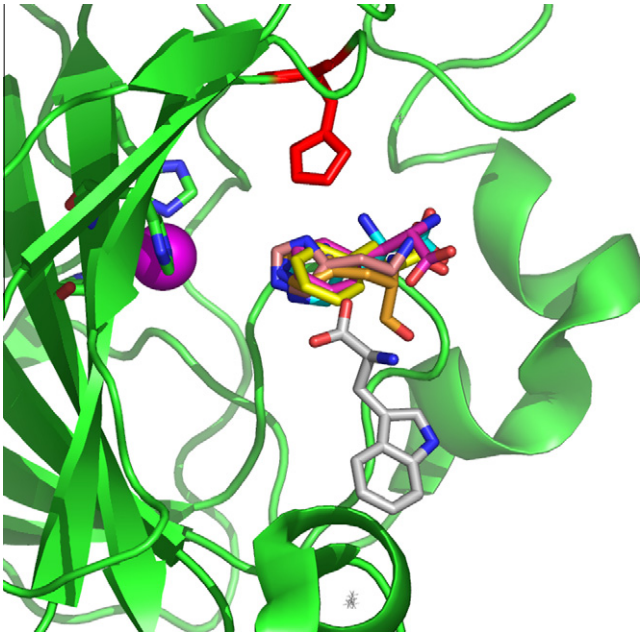


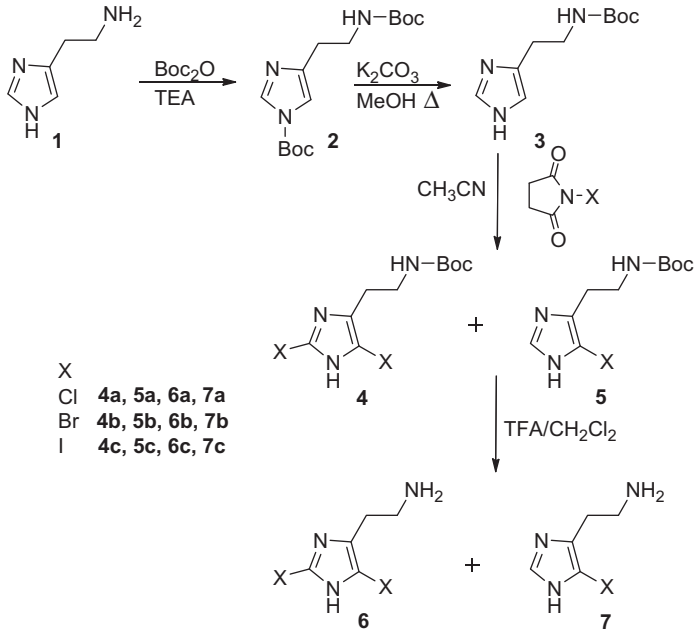
Figure 1. Superposition of the hCA II adducts with histamine (pink, PDB 1AVN)[11]; L-His (gold, PDB 2ABE)[9]; D-His (sky blue, PDB 2EZ7)[9]; L-Phe (magenta, PDB 2FMG)[10a]; D-Phe (yellow, PDB 2FMZ)[10b]; and D-Trp (grey, PDB file 3EFI).[10c] The Zn(II) ion from the enzyme active site is shown as the violet sphere, and is coordinated by three His residues (His94, 96 and 119 shown as blue and green sticks) and a water molecule/hydroxide ion (not shown). The natural proton shuttle residues, His64 is shown in red, whereas the protein backbone is the green ribbon.

not superposable to the other activators), in the activator binding site B (Fig. 1).[10c] Histamine **1**, which binds with the imidazole moiety orientated towards the middle of the active site cavity and the aminoethyl moiety towards its exit, was much used as a lead molecule to design both low molecular[7,8,14] and nanoparticle type CAAs.[18] Although histamine itself is a rather ineffective activator for hCA II (K_A of 125 μM),[11] many of its derivatives showed a better activity towards this isoform and also against hCA I, the slower cytosolic isozyme.[7,8,14,18] We have thus used histamine 1 as lead molecule to design novel activators targeting the cytosolic isoforms hCA I and II which we report here. Our goal was to prepare mono- and dihalogenated histamine derivatives (at the imidazole ring), since due to their highly electronegative character the halogen atoms usually induce a net change in the physico-chemical properties of the biologically active molecules containing them. Such halogenated histamine derivatives have not been tested up to now for their effects as CAAs.

The different mono and dihalogenated histamines investigated here were prepared by a four steps synthesis procedure, starting from commercially available histamine (Scheme 1). Histamine **1** was fully protected by the Boc group, both at the ethylamino and imidazole nitrogens, leading to **2**, which was thereafter selectively deprotected on the $N\tau$ of the imidazole ring in the presence of potassium carbonate in methanol.[19] The resulting key intermediate **3** was halogenated with *N*-halogenosuccinimides in acetonitrile, leading to a mixture of mono- and dihalogenated compound, of types **4** and **5**.[20] The difference of polarity between **4** and **5** allowed their facile separation by silica gel column chromatography. The final deprotection step of **4** and **5**, in the presence of trifluoroacetic acid (TFA) afforded the mono- and dihalogenated compounds **6** and **7** in quantitative yields.[21]

The halogenated protected and deprotected histamine derivatives **4–7** prepared as described above have been tested[22] for their activating properties against isoforms hCA I and II, which are among the most physiologically relevant and widely distributed CAs in mammals, including humans.[1–4] The following structure–activity relationship (SAR) can be observed from the data of Table 1:

(i) Against hCA I, the boc-protected halogenated derivatives of type **4** and **5**, were less effective than he lead **1** (KA of 2 μM),[11] with activation constants in the range of 5. 4–29.3 μM. It may be observed that for the dihalogenated derivatives **4** the activating effects diminished with the increase of the atomic weight of the halogen, whereas for the monohalogenated ones **5**, the reverse was true. However, the boc-deprotected compounds **6** and **7** showed highly



Scheme 1. Preparation of the halogenated histamine derivatives **4–7**.

Table 1
hCA I and II activation with histamine and halogenated/protected-histamine derivatives **4–7**, by a stopped-flow, CO_2 hydrase assay[22]

No.	X	K_A^* (μM)	
		hCA I	hCA II
Histamine		2.0	125
4a	Cl	5.4	50.2
4b	Br	7.1	44.5
4c	I	29.3	13.6
5a	Cl	16.5	24.8
5b	Br	13.3	28.5
5c	I	12.1	36.7
6a	Cl	0.021	0.115
6b	Br	0.015	0.096
6c	I	0.0009	0.065
7a	Cl	0.018	0.032
7b	Br	0.012	0.008
7c	I	0.0007	0.001

* Mean from three different determinations. Errors were in the range of ±10% of the reported values.

enhanced CA activating properties compared to the corresponding protected ones from which they were obtained and also compared to the lead **1**. Indeed, these CAAs showed activation constants in the range of 0.7–21 nM (Table 1). Comparable activity was observed both for the mono- as well as dihalogenated compounds. In all cases, the iodine derivatives were more active than the corresponding bromine ones which in turn were more active than the chlorinated histamines. Indeed, the di-iodinated histamine **6c** and the mono-iodinated one **7c** are among the most effective hCA I activators ever reported (K_As of 0.7–0.9 nM).

(ii) All compounds investigated here, of type **4–7**, were more potent as hCA II activators compared to histamine **1**, which is a quite weak activator of this isoforms (K_A of 125 μM).[11] The boc-protected compounds **4** and **5** were again less effective CAAs, with K_As in the range of 13.6–50.2 μM. For the dihalogenated compounds **4**, activity increased with the increase in the atomic weight of the halogen atom, whereas for the monohalogenated, boc-protected derivatives **5**, the most active compound was the chlorine derivative and the least active one the iodinated one. However, all of them are medium potency-weak hCA II activators. The deprotected derivatives **6** and **7** on the other hand showed an enhanced activating power, with K_As in the range of 1–115 nM (Table 1). The dihalogenated histamines **6a–c** showed activation constants of 65–115 nM, with activity increasing from chlorine to iodine-containing compounds. A further increase of activity has been observed for the monohalogenated derivatives **7**, which had K_As in the range 1–32 nM, with activity varying in the same manner with the nature of the halogen atom as for the dihalogenated ones. Thus, generally the best activity has been observed for compounds incorporating only one halogen atom, and the heavier this was, better the CA activating properties were.

In conclusion, we prepared ring mono- and dihalogenated histamine derivatives incorporating chlorine, bromine and iodine. The corresponding boc-protected derivatives (at the aminoethyl group) ere also obtained. All these histamines have been assayed for the activation of the cytosolic CA isoforms hCA I and II. Very net SAR has been obtained for these enzyme activators: against both isoforms the boc-protected histamines were moderately active, with activation constants of 5.4–29.3 μM against hCA I, and of 13.6–50.2 μM against hCA II. The boc-deprotected halogenated histamines showed a enhanced activity as CAAs, with activation constants of 0.7–21 nM against hCA I and of 1–115 nM against hCA II. The monohalogenated histamines were generally more active than the corresponding dihalogenated derivatives. CA activation increased with the increase of atomic weight of the halogen. Some of the compounds reported here are among the most effective hCA I and II activators reported so far, with low nanomolar or subnanomolar activation constants.

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21. *tert-Butyl 4-(2-(tert-butoxycarbonylamino)ethyl)-1H-imidazole-1-carboxylate* (**2**): Yield: 38%; R*f*: 0.38 (DCM 9.5/MeOH 0.5); mp: 129.5–130.5 °C; ^{1}H NMR (400 MHz, DMSO): δ ppm 8.09 (d, *J* = 1.2 Hz, 1H), 7.25 (d, *J* = 0.9 Hz, 1H), 6.85 (t, *J* = 5.5 Hz, 1H), 3.15 (dd, *J* = 13.1 Hz, *J* = 7.0 Hz, 2H), 2.57 (t, *J* = 7.1 Hz, 2H), 1.55 (s, 9H), 1.36 (s, 11H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155.59, 146.82, 140.97, 85.06, 83.00, 77.59. MS ESI$^+$/ESI$^-$: *m/z* 312.33 (M+H)$^+$, 310.04 (M−H)$^-$.
tert-Butyl 2-(1H-imidazol-4-yl)ethylcarbamate (**3**): Yield: 19%; R*f*: 0.19 (DCM 9/MeOH 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.44 (s, 1H), 6.78 (s, 1H), 6.75 (s, 2H), 6.71 (s, 1H), 3.79 (s, 6H), 3.69 (s, 2H), 2.86 (t, 2H, *J* = 6.5 Hz), 2.73 (t, 2H, *J* = 6.5 Hz). MS ESI$^+$: *m/z* 212.22 (M+H)$^+$.
tert-Butyl 2-(2,5-dichloro-1H-imidazol-4-yl)ethylcarbamate (**4a**): Yield: 40%; R*f*: 0.34 (DCM 9.5/MeOH 0.5); ^{1}H NMR (400 MHz, DMSO): δ ppm 7.95 (s, 1H), 6.92 (t, *J* = 5.8 Hz, 1H), 3.09 (m, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 1.35 (s, 9H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155.53, 126.59, 77.70, 38.89, 28.26, 24.35; MS ESI$^+$/ESI$^-$: *m/z* 280.3–282.29 (M+H)$^+$, 302.27–304.27 (M+Na)$^+$, 278.17–280.16 (M−H)$^-$.
tert-Butyl 2-(5-chloro-1H-imidazol-4-yl)ethylcarbamate (**5a**): Yield: 49%; R*f*: 0.18 (DCM 9.5/MeOH 0.5); ^{1}H NMR (400 MHz, DMSO): δ ppm 7.58 (s, 1H, H-1), 6.90 (t, *J* = 5.5 Hz, 1H), 3.14–3.05 (m, 2H), 2.63 (t, *J* = 7.3 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, DMSO): δ ppm 155.47, 133.21, 122.65, 77.60, 39.15, 28.22, 24.11; MS ESI$^+$/ESI$^-$: *m/z* 246.31–248.30 (M+H)$^+$, 244.31–246.30 (M−H)$^-$.
2-(2,5-Dichloro-1H-imidazol-4-yl) ethanamine (**6a**): Yield: 100%; ^{1}H NMR (400 MHz, DMSO): δ ppm 11.07 (s, 1H), 7.99 (t, *J* = 5.7 Hz, 1H), 3.06–2.96 (m, 2H), 2.81 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 136.51, 127.29, 122.36, 37.51, 22.10; MS ESI$^+$: *m/z* 180.15–182.14 (M+H)$^+$, 202.19–204.24 (M+Na)$^+$.
2-(5-Chloro-1H-imidazol-4-yl)ethanamine (**7a**): Yield: 100%; ^{1}H NMR (400 MHz, DMSO): δ ppm 7.97 (s, 1H), 3.09–2.97 (m, 2H), 2.87 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 121.77, 117.24, 37.64, 21.72; MS ESI$^+$: *m/z* 146.13–148.18 (M+H)$^+$.
tert-Butyl 2-(2,5-dibromo-1H-imidazol-4-yl)ethylcarbamate (**4b**): Yield: 37%; R*f*: 0.38 (DCM 9.5/MeOH 0.5); mp: 100–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 11.06 (s, 1H), 6.92 (t, *J* = 5.6 Hz, 1H), 3.09 (dd, *J* = 13.2 Hz, *J* = 5.8 Hz, 2H), 2.58 (t, *J* = 13.3 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155,47, 77.62, 38.96, 28.25, 25.04; MS ESI$^+$/ESI$^-$: *m/z* 389.42–391.38 (M+Na)$^+$, 366.12–368.10–370.07 (M−H)$^-$.
tert-Butyl 2-(5-bromo-1H-imidazol-4-yl)ethylcarbamate (**5b**): Yield: 50%; R*f*: 0.16 (DCM 9.5/MeOH 0.5); mp: 53–55 °C; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.32 (s, 1H), 7.55 (s, 1H), 6.92 (t, *J* = 5.6 Hz, 1H), 3.14–2.98 (m, 2H), 2.61 (t, *J* = 7.4 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155.49, 134.73, 77.63, 38.88, 28.27, 24.82; MS ESI$^+$/ESI$^-$: *m/z* 290.25–292.24 (M+H)$^+$, 312.18–313.29 (M+Na)$^+$, 289.33–290.32 (M−H)$^-$.
2-(2,5-Dibromo-1H-imidazol-4-yl)ethanamine (**6b**): Yield: 100%; mp: 165–167 °C; ^{1}H NMR (400 MHz, DMSO): δ ppm 11.08 (s, 1H), 7.96 (s, 2H), 3.10–2.93 (m, 2H), 2.80 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 114.86, 37.60, 24.04; MS ESI$^+$: *m/z* 268.17–270.19–272.15 (M+H)$^+$
2-(5-Bromo-1H-imidazol-4-yl)ethanamine (**7b**): Yield: 100%; ^{1}H NMR (400 MHz, DMSO): δ ppm 10.28 (s, 1H), 8.42 (s, 1H), 7.98 (s, 2H), 3.12–2.98 (m, 2H), 2.89 (t, *J* = 13.6 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 135.43, 125.89, 107.35, 37.52, 22.23; MS ESI+: *m/z* 190.15–192.20 (M+H)$^+$.
tert-Butyl 2-(2,5-diiodo-1H-imidazol-4-yl)ethylcarbamate (**4c**): Yield: 35%; R*f*: 0.48 (DCM 9.5/MeOH 0.5); mp: 68–70 °C; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.76 (s, 1H), 6.90 (t, *J* = 5.6 Hz, 1H), 3.04–3.09 (m, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155.44, 136.18, 77.60, 40.15, 28.23, 26.04; MS ESI$^+$/ESI$^-$: *m/z* 464.12 (M+H)$^+$, 486.12 (M+Na)$^+$, 462.20 (M−H)$^-$.
tert-Butyl 2-(5-iodo-1H-imidazol-4-yl)ethylcarbamate (**5c**): Yield: 38%; R*f*: 0.26 (DCM 9.5/MeOH 0.5); mp: 55–57 °C; ^{1}H NMR (400 MHz, DMSO): δ ppm 12.27 (s, 1H), 7.58 (s, 1H), 6.92 (t, *J* = 5.5 Hz), 3.07 (m, 2H), 2.60 (t, *J* = 7.4 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO): δ ppm 155.37, 130.19, 77.62, 40.01, 28.30, 25.96; MS ESI$^+$/ESI$^-$: *m/z* 338.36 (M+H)$^+$, 336.34 (M−H)$^-$.
2-(2,5-Diiodo-1H-imidazol-4-yl)ethanamine (**6c**): Yield: 100%; ^{1}H NMR (400 MHz, DMSO): δ ppm 11.08 (s, 1H), 7.88 (s, 2H), 2.99 (m, 2H), 2.78 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 137.58, 120.16, 37.99, 24.11; MS ESI$^+$: *m/z* 364.10 (M+H)$^+$.
2-(5-Iodo-1H-imidazol-4-yl) ethanamine (**7c**): Yield: 100%; ^{1}H NMR (400 MHz, DMSO): δ ppm 9.07 (s, 1H), 8.05 (s, 2H), 3.14–3.02 (m, 2H), 2.93 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO): δ ppm 137.75, 120.37, 37.60, 23.25; MS ESI$^+$: *m/z* 238.15 (M+H)$^+$.
22. Khalifah, R.G. *J. Biol. Chem.* **1971**, *246*, 2561. An applied photophysics stopped-flow instrument was used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant ionic strength), following the CA-catalyzed CO_2 hydration reaction for a period of 10 s at 25 °C. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and activation constants. For each activator 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 activators (10 mM) were prepared in distilled-deionized water and dilutions up to 0.1 nM were done thereafter with distilled-deionized water. Activator and enzyme solutions were preincubated together for 15 min (standard assay at room temperature, or for prolonged periods of 24–72 h, at 4 °C) prior to assay, in order to allow for the formation of the E–A complex. The activation constant (K_A), defined similarly with the inhibition constant K_I, can be obtained by considering the classical Michaelis–Menten equation (Eq. 1), which has been fitted by nonlinear least squares by using PRISM 3:

$$v = v_{max}/\{1 + K_M/[S](1 + [A]_f/K_A)\} \quad (1)$$

where $[A]_f$ is the free concentration of activator.
Working at substrate concentrations considerably lower than K_M ($[S] \ll K_M$), and considering that $[A]_f$ can be represented in the form of the total concentration of the enzyme ($[E]_t$) and activator ($[A]_t$), the obtained competitive steady-state equation for determining the activation constant is given by Eq. 2:[14,18]

$$v = v_0.K_A/\{K_A + ([A]_t - 0.5\{([A]_t + [E]_t + K_A) - ([A]_t + [E]_t + K_A)^2 - 4[A]_t.[E]t)^{1/2}\}\} \quad (2)$$

where v_0 represents the initial velocity of the enzyme-catalyzed reaction in the absence of activator.[14,18]