Famotidine, an Antiulcer Agent, Strongly Inhibits *Helicobacter pylori* and Human Carbonic AnhydrasesAndrea Angeli,[†] Marta Ferraroni,[‡] and Claudiu T. Supuran^{*,†}[†]University of Florence, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy[‡]University of Florence, Department of Chemistry “Ugo Schiff”, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Florence, Italy

Supporting Information

ABSTRACT: Famotidine, an antiulcer drug incorporating a sulfamide motif, was investigated as carbonic anhydrase inhibitor (CAI). It acts as a nanomolar inhibitor of several human (hCA II, VI, VII and XII) and *Helicobacter pylori* CAs. The high resolution X-ray structures of famotidine bound to hCA I and II revealed interesting aspects related to its CA inhibition mechanism, offering the possibility to develop antibacterials with a novel mechanism of action.

KEYWORDS: Carbonic anhydrase, famotidine, *Helicobacter pylori*, inhibitor, metalloenzymes

The third generation histamine H_2 receptor antagonists such as cimetidine, ranitidine, and famotidine (Figure 1)

Figure 1. Third generation of H_2 antagonists in clinical use.

are widely used for the treatment of duodenal and gastric ulcers, gastroesophageal reflux disease, and pathological hypersecretory conditions such as Zollinger–Ellison syndrome.^{1–3} Famotidine incorporates a sulfamide moiety, a bioisostere of the sulfonamide group, a well-known zinc binding group present in carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs).⁴ However, the effects of famotidine on these zinc metallo-enzymes was scarcely investigated until now.⁵ CAs are ubiquitous metallo-enzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate and protons.^{6,7} In humans, 15 different α -isoforms are present, with a very different subcellular localization and tissue distribution.⁸ There are several cytosolic isoforms (hCA I–III, VII, XIII), four membrane-bound isozymes (CA IV, IX, XII, and XIV), two mitochondrial ones (CA VA, VB), and a secreted CA isozyme, CA VI, apart from three acatalytic ones (CA VIII, X and XI).⁸

These enzymes play crucial physiological/pathological roles in acid–base homeostasis, secretion of electrolytes, transport

of ions, by providing bicarbonate and by regulating the pH in the gastric acid production, within the stomach parietal cells, as well as bile production, pancreatic juice production, intestinal ion transport, and protection of gastrointestinal tract from extreme pH conditions (too acidic or too basic).^{1,9,10} In this particular context, we decided to investigate whether famotidine shows CA inhibitory effects. All catalytically active human CA isoforms (hCA I–XIV), as well as the two CAs present in the bacterial pathogen *Helicobacter pylori* (α -HpCA, β -HpCA), the etiologic agent responsible of gastric ulcers, were included in the study. Furthermore, we report the X-ray structure of famotidine in complex with hCA I and hCA II, allowing us to unravel interesting aspects related to its CA inhibition mechanism. The inhibition profiles of famotidine and acetazolamide (AAZ), as standard sulfonamide inhibitor against all catalytically active CA isoforms is shown in Table 1. The cytosolic isoforms had a very different inhibition profile with famotidine. hCA VII was the most susceptible isoform to inhibition with this drug, with an inhibition constant K_i of 3.0 nM. However, the two widespread cytosolic isoforms hCA I and hCA II showed a very different inhibition with this compound: hCA II was effectively inhibited (K_i 57.9 nM), whereas hCA I was more than ten times less sensitive to famotidine inhibition compared to hCA II, with a K_i of 922.4 nM. Famotidine was a moderate inhibitor of isoform hCA XIII (K_i 171.5 mM) and lost any activity against hCA III, with a K_i

Received: July 24, 2018

Accepted: September 4, 2018

Published: September 4, 2018

Table 1. hCA I–XIV Inhibition Data with Famotidine and Acetazolamide (AAZ) as a Standard Inhibitor, by a CO₂ Hydration, Stopped-Flow Technique¹¹

cmpd	K_i (nM) ^a	
	famotidine	AAZ
hCA I	922.4	250.0
hCA II	57.9	12.1
hCA III	>10000	>10000
hCA IV	938.8	74.0
hCA VA	1429.5	63.0
hCA VB	5328.2	54.0
hCA VI	98.2	11.0
hCA VII	3.0	2.5
hCA IX	126.3	25.8
hCA XII	45.3	5.7
hCA XIII	171.5	17.0
hCA XIV	677.2	41.0

^aMean from three different assays, by a stopped-flow technique (errors were in the range of ± 5 –10% of the reported values).

> 10 μ M. The two mitochondrial isoforms (hCA VA and VB) were poorly inhibited by this sulfamide drug, in the micromolar range (K_i s of 1.4 and 5.3 μ M, respectively). The membrane-bound isoforms, similar to the cytosolic ones discussed above, showed a heterogeneous inhibition pattern. hCA XII, a tumor associated isoform and recently validated as antitumor target, was effectively inhibited by famotidine with a K_i of 45.3 nM. The second enzyme overexpressed in hypoxic tumors, hCA IX, was, however, less inhibited by famotidine, with a K_i of 126.3 nM (Table 1). The last two membrane-associated isoforms hCA IV and XIV, instead, were weakly inhibited by this drug, in the high nanomolar range (K_i 938.8 and 677.2 nM).

H. pylori, a Gram-negative neutralophile, was shown to be the cause of chronic gastritis, peptic ulcers, and, more recently, gastric cancer, the second most common tumor in humans.^{12,13} This gastric pathogen encodes for two classes of CAs, an α - and a β -one. Both enzymes are crucial for its survival in the acidic environment within the stomach, and recent studies showed that sulphonamides block the growth of the pathogen *in vitro* and *in vivo*.^{14–16} Thus, in case of interference with these enzymes, famotidine might be used as a new pharmacologic tool in the management of drug-resistant *H. pylori*.¹⁷ For this reason, we performed inhibition studies of the two bacterial CAs (Table 2). The α -class enzyme was effectively inhibited by this compound, with an inhibition constant of 20.7 nM, comparable to clinically used sulphonamide acetazolamide. However, *hp* β CA was less effectively inhibited by famotidine (K_i of 49.8 nM). However, also for this enzyme the activity was comparable to AAZ. An

Table 2. *hp* α CA and *hp* β CA Inhibition Data with Famotidine and Acetazolamide (AAZ) as a Standard Inhibitor, by a CO₂ Hydration, Stopped-Flow Technique¹¹

cmpd	K_i (nM) ^a	
	famotidine	AAZ
<i>hp</i> α CA	20.7	21.4
<i>hp</i> β CA	49.8	40.0

^aMean from three different assays, by a stopped-flow technique (errors were in the range of ± 5 –10% of the reported values).

interesting observation was that famotidine proved to be more active against *H. pylori* enzymes compared to the two human dominant cytosolic isoforms (hCA I and hCA II), whereas AAZ was an effective inhibitor for both of them (Table 1).

Considering the abundant localization in the gastrointestinal tract of the cytosolic hCA I and II, as well as their crucial roles in pH regulation, we obtained the high-resolution X-ray crystal structures of the adducts of famotidine with these enzymes, in order to understand in detail the binding modes of the drug. Inspection of the initial $F_o - F_c$ electron density maps of both active sites showed well-defined electron density, fully compatible with the presence of famotidine, and, surprisingly, revealed two possible conformation of the drug for both adducts (Figure 2A,B). Famotidine coordinates the catalytic

Figure 2. Active site region of hCA I/famotidine (A) and hCA II/famotidine (B) complexes. Inhibitors shown as σ_A -weighted $|F_o - F_c|$ density maps at 2.0σ . The zinc ion (gray sphere) and its three His ligands are shown in CPK color.

zinc ion of the two CA isoforms by means of one nitrogen atom of the sulfamide group, displacing the zinc bound water molecule/hydroxide ion, resulting in a tetrahedral coordination geometry of the metal ion, similarly to other sulfamide and sulfonamide compounds.^{4,18}

The hCA I/famotidine complex showed two different orientations of the inhibitor (Figure 3). The first one, labeled in green, exhibited different hydrogen bonds stabilizing the enzyme–inhibitor adduct, such as the ones involving the guanidine tail and Asn69 or the nitrogen atom near the

Figure 3. Active site region of hCA I/famotidine adduct. Inhibitor shown as σ_A -weighted $|F_o - F_c|$ density map at 2.0σ . Hydrogen bonds, van der Waals interactions, and the active site Zn^{2+} -ion coordination are also shown.

sulfamide moiety and Thr199 and His200. However, hydrophobic interactions were almost nonexistent in this complex. We found only one such interaction among Leu198 and the methylene chain of famotidine. The opposite orientation of the second structure (labeled in magenta), surprisingly, did not show any interaction with side chains of the protein (Figure 3). A completely different situation was observed when the hCA II active site was analyzed. Again, two possible orientations of famotidine were observed, both located in the hydrophobic region delimited by a small pocket aligned by the side chains of Phe131, Leu198, and Pro201 (Figure 4). The thiazole ring of

Figure 4. Active site region of hCA II/famotidine adduct. Inhibitor shown as σA -weighted $|F_o - F_c|$ density map at 2.0σ . Hydrogen bonds, van der Waals interactions, and the active site Zn^{2+} -ion coordination are also shown.

one famotidine orientation (labeled in green) formed strong hydrophobic interactions with these residues and a water bridge with Pro201 by means of the azomethine nitrogen atom. This portion of the molecule was oriented differently in two conformations: in one, the nitrogen atom participates in several hydrogen bonds with Thr199 and Thr200. In the second one, a reverse orientation of the nitrogen was seen, with no interactions with the protein. Finally, the sulfamide oxygen is involved in a bifurcated hydrogen bond with Thr200 (Figure 4). Electron density was absent for the guanidine tail of the conformation labeled in magenta in Figure 4 and was not introduced in the complex model.

The structural superimposition between the hCA I and hCA II complexes is reported in Figure 5, which shows that, while the sulfamide moieties of famotidine are rather well superimposable, the two aliphatic tails present different orientations and, as a consequence, diverse interactions with amino acid side chains from the two active sites. Phe131 in hCA II proved

Figure 5. Structural superimposition of famotidine/hCA I and famotidine/hCA II complexes. hCA I and hCA II adducts are colored in green and magenta, respectively.

to be essential to collocate famotidine within the lipophilic side of the active site cavity, which strongly correlates with the inhibition potency of this drug against hCA II. However, the presence of Leu131 in hCA I led to a loose van der Waals interaction, which did not force famotidine toward the lipophilic side of the active site, leading thus to two opposite orientations of the scaffold and few hydrophobic interactions with the active site. These characteristics reflect the loss of inhibitory potency against this isoform compared to hCA II.

In summary, we have analyzed the inhibition pattern of famotidine against all human active CAs. The drug shows particular efficacy for the cytosolic isoforms hCA II and hCA VII. Furthermore, the two class bacterial enzymes from *H. pylori* (hpaCA and hpβCA) were also highly inhibited by this drug, raising thus the possibility that the effective antiulcer effects of famotidine may be due not only to its action of H₂-receptor antagonist but also due to the fact that, by inhibiting the bacterial CAs, the survival of the pathogen within the gastric niche is compromised, as demonstrated earlier for acetazolamide.¹⁹ Indeed, this compound was used as an antiulcer agent with some success, but its side effects due to inhibition of CAs in other tissues than the stomach precluded its large clinical use.²⁰ Famotidine is a much more isoform-selective CAI compared to acetazolamide and effectively inhibits the bacterial CAs too, allowing us to propose it as a new lead for the design of anti-infectives with a multifactorial mechanism of action.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00334.

Synthetic procedures, characterization of compounds, in vitro kinetic procedure, X-ray crystallography, and biological assay (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +39-055-4573729. E-mail: claudiu.supuran@unifi.it.

ORCID

Marta Ferraroni: 0000-0001-7258-738X

Claudio T. Supuran: 0000-0003-4262-0323

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CAs, carbonic anhydrases; AAZ, acetazolamide

■ REFERENCES

- (1) Sakurai, K.; Nagahara, A.; Inoue, K.; Akiyama, J.; Mabe, K.; Suzuki, J.; Habu, Y.; Araki, A.; Suzuki, T.; Satoh, K.; Nagami, H.; Harada, R.; Tano, N.; Kusaka, M.; Fujioka, Y.; Fujimura, T.; Shigeto, N.; Oumi, T.; Miwa, J.; Miwa, H.; Fujimoto, K.; Kinoshita, Y.; Haruma, K. Efficacy of omeprazole, famotidine, mosapride and teprenone in patients with upper gastrointestinal symptoms: an omeprazole-controlled randomized study. *BMC Gastroenterol.* **2012**, *12*, 42.

- (2) Howden, C. W. Clinical pharmacology of omeprazole. *Clin. Pharmacokinet.* **1991**, *20*, 38.
- (3) Ahmadi, A.; Ebrahimzadeh, M. A.; Ahmad-Ashrafi, S.; Karami, M.; Mahdavi, M. R.; Saravi, S. S. Hepatoprotective, antinociceptive and antioxidant activities of cimetidine, ranitidine and famotidine as histamine H₂ receptor antagonists. *Fundam. Clin. Pharmacol.* **2011**, *25*, 72.
- (4) De Simone, G.; Pizika, G.; Monti, S. M.; Di Fiore, A.; Ivanova, J.; Vozny, I.; Trapencieris, P.; Zalubovskis, R.; Supuran, C. T.; Alterio, V. Hydrophobic substituents of the phenylmethylsulfamide moiety can be used for the development of new selective carbonic anhydrase inhibitors. *BioMed Res. Int.* **2014**, *2014*, 523210.
- (5) Demir, Y.; Nadaroğlu, H.; Demir, N. Effects of omeprazole, famotidine, and ranitidine on the enzyme activities of carbonic anhydrase from bovine stomach in vitro and rat erythrocytes in vivo. *Biol. Pharm. Bull.* **2004**, *27*, 1730.
- (6) Supuran, C. T. Structure and function of carbonic anhydrases. *Biochem. J.* **2016**, *473*, 2023.
- (7) Supuran, C. T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discovery* **2008**, *7*, 168.
- (8) Supuran, C. T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin. Drug Discovery* **2017**, *12*, 61.
- (9) Lönnerholm, G.; Selking, O.; Wistrand, P. J. Amount and distribution of carbonic anhydrases CA I and CA II in the gastrointestinal tract. *Gastroenterology* **1985**, *88*, 1151.
- (10) Carter, M. J.; Parsons, D. S. The isoenzymes of carbonic anhydrase: tissue, subcellular distribution and functional significance, with particular reference to the intestinal tract. *J. Physiol.* **1971**, *215*, 71.
- (11) Khalifah, R. G. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. *J. Biol. Chem.* **1971**, *246*, 2561.
- (12) Nishimori, I.; Minakuchi, T.; Kohsaki, T.; Onishi, S.; Takeuchi, H.; Vullo, D.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: the beta-carbonic anhydrase from *Helicobacter pylori* is a new target for sulfonamide and sulfamate inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3585.
- (13) Nishimori, I.; Vullo, D.; Minakuchi, T.; Morimoto, K.; Onishi, S.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: DNA cloning and inhibition studies of the alpha-carbonic anhydrase from *Helicobacter pylori*, a new target for developing sulfonamide and sulfamate gastric drugs. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2182.
- (14) Maresca, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. Inhibition of the alpha- and beta-carbonic anhydrases from the gastric pathogen *Helicobacter pylori* with anions. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 388.
- (15) Capasso, C.; Supuran, C. T. Bacterial, fungal and protozoan carbonic anhydrases as drug targets. *Expert Opin. Ther. Targets* **2015**, *19*, 1689.
- (16) Modak, J. K.; Liu, Y. C.; Supuran, C. T.; Roujeinikova, A. Structure-Activity Relationship for Sulfonamide Inhibition of *Helicobacter pylori* α -Carbonic Anhydrase. *J. Med. Chem.* **2016**, *59*, 11098.
- (17) Amin, M.; Iqbal, M. S.; Hughes, R. W.; Khan, S. A.; Reynolds, P. A.; Enne, V. I.; Mirza, A. S. Mechanochemical synthesis and in vitro anti-*Helicobacter pylori* and urease inhibitory activities of novel zinc(II)-famotidine complex. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 383.
- (18) Di Fiore, A.; De Simone, G.; Alterio, V.; Riccio, V.; Winum, J. Y.; Carta, F.; Supuran, C. T. The anticonvulsant sulfamide JNJ-26990990 and its S,S-dioxide analog strongly inhibit carbonic anhydrases: solution and X-ray crystallographic studies. *Org. Biomol. Chem.* **2016**, *14*, 4853.
- (19) Marcus, E. A.; Moshfegh, A. P.; Sachs, G.; Scott, D. R. The periplasmic alpha-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. *J. Bacteriol.* **2005**, *187*, 729.
- (20) Buzás, G. M.; Supuran, C. T. The history and rationale of using carbonic anhydrase inhibitors in the treatment of peptic ulcers. In memoriam Ioan Pușcaș (1932–2015). *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 527.