Carbonic Anhydrase Inhibitors: DNA Cloning and Inhibition Studies of the α-Carbonic Anhydrase from *Helicobacter pylori,* A New Target for Developing Sulfonamide and Sulfamate Gastric Drugs†

Isao Nishimori,‡ Tomoko Minakuchi,‡ Kaori Morimoto,‡ Shuichi Sano,‡ Saburo Onishi,‡ Hiroaki Takeuchi,§ Daniela Vullo,|| Andrea Scozzafava,|| and Claudiu T. Supuran*,||

Departments of Gastroenterology and Hepatology and of Laboratory Medicine, Kochi Medical School, Nankoku, Kochi 783-8505, Japan, and Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Room 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy

Received December 19, 2005

We have cloned and sequenced *Helicobacter pylori* α-class carbonic anhydrase (hpCA) from patients with different gastric mucosal lesions, including gastritis ($n = 15$), ulcer ($n = 6$), and cancer ($n = 16$). Although several polymorphisms were newly identified such as ^{12}Ala, ^{13}Thr, ^{16}Ile, and ^{168}Phe, there was no significant relevance of any polymorphism with gastric mucosal lesion types. A library of sulfonamides/sulfamates has been investigated for the inhibition of hpCA, whereas new derivatives have been obtained by attaching 4-*tert*-butyl-phenylcarboxamido/sulfonamido tails to benzenesulfonamide/1,3,4-thiadiazole-2-sulfonamide scaffolds. All types of activity for inhibition of hpCA have been detected. Dorzolamide and simple 4-substituted benzenesulfonamides were weak inhibitors (K_I 873−4360 nM). Sulfanilamide, orthanilamide, some of their derivatives, and indisulam showed better activity (K_I 413−640 nM), whereas most of the clinically used inhibitors, such as methazolamide, ethoxzolamide, dichlorophenamide, brinzolamide, topiramate, zonisamide, etc., acted as medium-potency inhibitors (K_I 105−378 nM). Some potent hpCA inhibitors were detected too (K_I 12−84 nM) among acetazolamide, 4-amino-6-chloro-1,3-benzenedisulfonamide and some newly designed compounds incorporating lipophilic tails. Some of the newly prepared derivatives had selectivity ratios for inhibiting hpCA over hCA II in the range of 1.25−3.48, showing thus some selectivity for inhibiting the bacterial enzyme. Since hpCA is essential for the survival of the pathogen in acid, it might be used as a new pharmacologic tool in the management of drug-resistant *H. pylori*.

Introduction

The α-carbonic anhydrases (CAs, EC 4.2.1.1) constitute a family of monomeric zinc metalloenzymes that catalyze the reversible hydration of CO_2 to bicarbonate and a proton.[1−5] We have been working with the molecular cloning of some of the 16 presently known human CA (hCA) isoforms,[6−8] as well as screening analyses for inhibitory effects of a variety of compounds on most of them, showing that various such isozymes (e.g., hCA II, IV, VA, VB, VII, IX, XII, XIII, and XIV) constitute valid targets for the development of novel antiglaucoma, antitumor, antiobesity, or anticonvulsant drugs.[9−13] Furthermore, very recently representatives of the α- or β-CA class have been cloned and characterized in other organisms, such as *Plasmodium falciparum*,[14] *Mycobacterium tuberculosis*,[15] *Cryptococcus neoformans*,[16] or *Candida* spp.,[17] some of them being also investigated from the inhibition point of view,[14,16] as it has been proved that these enzymes are critical for the growth or virulence of these pathogens.[14−17] Since many of these organisms are highly pathogenic and present different degrees of resistance to the currently available drugs targeting them, inhibition of their CAs may constitute novel approaches to fighting such diseases.[14−17]

Helicobacter pylori, a Gram-negative neutralophile discovered by Warren and Marshall in the early 1980s,[18] was shown to be associated with chronic gastritis, peptic ulcers, and, more recently, gastric cancer, the second most common tumor in humans.[19] *H. pylori* is a globaly spread pathogen with roughly 50% of the human population being contaminated, causing sometimes severe gastrointestinal diseases that lead to a significant morbidity and mortality.[20] Although there is an effective treatment for peptic ulcer disease caused by *H. pylori*, usually consisting of a triple therapy with two antibiotics (amoxicillin and clarithromycin) and a proton pump inhibitor (PPI), such first-line treatment/eradication regimens are constantly being compromised by an increase in the prevalence of antibiotic resistance.[21−23] After failure of the eradication by the first-line treatment, an empirical quadruple regimen (PPI, bismuth, tetracycline, and metronidazole) has generally been used as the second-line therapy. However, several studies have demonstrated that even two consecutive regimens failed to eradicate *H. pylori* in some patients.[21−23] Thus, there is a real need for the development of alternative therapies, eventually exploiting novel targets, that should be devoid of the problems arising with currently available drugs.[23b]

H. pylori has the unique ability among bacteria to grow and multiply in the stomach, in the harsh and highly acidic conditions at pH values as low as 1.4.[24] Therefore, the pathogen has evolved in specialized processes that maintain the cytoplasmic pH around 6.4 for survival and growth. Basically, at least two enzymes are involved in these processes: urease[24] in the cytoplasm, and an α-CA (from now on designated as hpCA) in the periplasm,[24,25] which separates an outer membrane and

† Nucleotide sequences reported in this paper have been deposited with GenBank under Accession Number AB242859.

* Correspondence author: tel +39-055-457 3005; fax +39-055-4573385; e-mail claudiu.supuran@unifi.it.

‡ Department of Gastroenterology and Hepatology, Kochi Medical School.

§ Department of Laboratory Medicine, Kochi Medical School.

|| Università degli Studi di Firenze.

10.1021/jm0512600 CCC: $33.50
Published on Web 02/16/2006

an inner membrane of this bacterium. However, a β-CA has also been found in the cytoplasm of *H. pylori*, where it seems to play an important role in the urea and bicarbonate metabolism, as well as acid resistance of the pathogen.[26]

hpCA was cloned and purified in 2001 by Lindskog's group,[27] who showed that the enzyme had a catalytic activity similar to that of the human slow isoform hCA I (highly abundant in red blood cells and the gastrointestinal tract)[1−3] and that the enzyme was susceptible to inhibition by sulfonamides (and thiocyanate). However, no quantitative data were provided for acetazolamide inhibition of hpCA in this study.[27] Recently, Sachs' group[24,25] has proved that hpCA is essential for the acid acclimation and survival of the pathogen. In their elegant study,[25] using CA deletion mutants of *H. pylori* as well as the potent, clinically used sulfonamide inhibitor acetazolamide AAZ, it has been shown that the urease generation of NH_3 has a major role in regulation of the periplasmic pH and inner membrane potential under acidic conditions, allowing adequate bioenergetics necessary for survival and growth of the pathogen. In addition to urease, interestingly, hpCA was also shown to be crucial to such processes. Thus, Western analysis confirmed that hpCA was bound to the inner membrane, being present only within the periplasm of *H. pylori*. Furthermore, in the deletion mutant (i.e., *H. pylori* lacking the hpCA) or in the wild-type organism in the presence of acetazolamide, there was an approximately 1000-fold decrease in acid survival of *H. pylori*. It has also been proved that, in acid medium, the absence of hpCA activity decreased membrane integrity, as observed by using membrane-permeant and -impermeant fluorescent DNA dyes.[25] The increase in membrane potential and cytoplasmic buffering following urea addition to the wild-type organisms in acid was, on the other hand, absent in the hpCA knockout mutant and in the presence of acetazolamide, although the urease remained fully functional.[25] Thus, buffering of the periplasm to a pH consistent with viability depends not only on the ammonia efflux from the cytoplasm (and thus urease) but also on the conversion of CO_2 (produced by urease) to bicarbonate by the periplasmic hpCA.[25] In fact, the pK_a of the carbonic acid/bicarbonate couple of around 6.1 is very appropriate for such a task, unlike the ammonium/ammonia buffer, which, having a pK_a of 9.2, is less useful for buffering the periplasm to pH values close to neutrality. This excellent study[25] is in fact the proof-of-concept one that hpCA may be an attractive drug target for developing anti-*H. pylori* agents, provided that potent (and hopefully specific) inhibitors can be found.

In the present study we sequenced hpCA DNAs from a large panel of independent strains of *H. pylori*, which were obtained from patients with a variety of gastric mucosal lesions. We were interested to investigate whether the enzyme isolated from gastritis, gastric ulcer, and gastric cancer patients is the same or different. Furthermore, we evaluated the inhibitory effects of a panel of sulfonamides/sulfamates (known inhibitors of other α-CAs)[28] against this enzyme, showing that effective inhibitors targeting this bacterial CA can be detected/designed.

Results

Sequence of *H. pylori* α-CA. All DNA sequences of the hpCA obtained from 37 different *H. pylori* strains encoded a 247-amino acid polypeptide, which was similar to the clones reported by ASTRA research center[29] and Chirica et al.[27] Although all sequences encoded identical-length polypeptides, they showed a variety of point mutations, resulting in six types of amino acid substitutions (Figure 1). Figure 1 shows the aligned amino acid sequences of a clone (KMT-12), which contains all substitutions newly discovered in the present study, together with the sequences of three clones previously reported.[27,29] The novel amino acid substitutions include ^{10}Ala(GCG) → Val(GTG), ^{12}Thr(ACG) → Ala(GCA),^{13}Thr(GCT) → Ala(ACT), and ^{74}Ala(GCT) → Thr(ACT). ^{168}Leu(CTT) → Phe(TTT) was previously reported in the clone HP1186 by the TIGR sequence center.[30] It is noteworthy that only one strain (KMT-45) showed an identical amino acid sequence to that reported by Chirica et al.,[27] including ^{16}Ile(ATA) → Val(GTA). In total, six types of amino acid substitutions were deposited (Table 1), probably proving the high selection pressure under which the pathogen is found due to the drug treatment of patients from which the samples were obtained.

```
            1        * **  *(10/12/13/16)                    50
KMT-12      MKKTFLIALVLATSLIGAENTKWDYKNKENGPHRWDKLHKDFEVCKSGKS
26695-BBA   ---------A-TA--V-----------------------------------
J-99-ASTRA  ---------A-TA-----------------------------------
26695-TIGR  ---------A-TA-----------------------------------

 51                      *(74)                                 110
  QSPINIEHYYHTQDKADLQFKYATSKPKAVFFTHHTLKASFEPTNHINYRGHDYVLDNVH
  ----------------------A-----------------------------------
  ----------------------A-----------------------------------
  ----------------------A-----------------------------------

111                                                  (168)* 170
  FHAPMEFLINNKTRPLSAHFVHKDAKGRLLVLAIGFEEGKENPNLDPILEGIQKKQNFKE
  ------------------------------------------------------------L--
  ------------------------------------------------------------L--
  ------------------------------------------------------------

171             Δ(186)                                        230
  VALDAFLPKSINYYHFNGSLTAPPCTEGVAWFVIEEPLEVSAKQLAEIKKRMKNSPNQRP
  ------------------------------------------------------------
  ------------------------------------------------------------
  --------------LTALSPLLLAQRGSWHGL(end)

231              247
  VQPDYNTVIIKSSAETR
  -----------------
```

Figure 1. Alignment of amino acid sequence of α-CA from four *H. pylori* strains. *H. pylori* strain KMT-12 was obtained from a Japanese patient with gastritis and contains all substitutions newly discovered in the present study as compared to the sequences of three clones previously reported.[27,29−31] Strain 26695 was isolated from a patient with gastritis in the United Kingdom.[31] From this strain, two different sequences for hpCA were reported, one by Chirica et al. (26695-BBA)[27] and one by The Institute for Genomic Research (TIGR) sequence center (26695-TIGR).[30,31] Strain J99 was isolated from a patient with a duodenal ulcer in the United States,[29] and the sequence was deposited by ASTRA sequence center (J-99-ASTRA).[29] Polymorphism with amino acid number is indicated by asterisks and parentheses above the sequences. A reading frame shift at ^{186}Phe occurred in 26695-TIGR (△), resulting an early appearance of the stop codon in the TIGR clone.

Table 1. Polymorphisms in α-CA Sequence of *H. pylori* from Japanese Patients with a Variety of Gastric Mucosal Lesions

residue no.	amino acid	gastritis (n = 15; %)	gastric ulcer (n = 6; %)	gastric cancer (n = 16; %)	total (n = 37; %)
10	Ala	10 (67)	5 (83)	12 (75)	27 (73)
10	Val	5 (33)	1 (17)	4 (25)	10 (27)
12	Thr	1[a] (7)	0 (0)	0 (0)	1 (3)
12	Ala	14 (93)	6 (100)	16 (100)	36 (97)
13	Ala	1[a] (7)	0 (0)	0 (0)	1 (3)
13	Thr	14 (93)	6 (100)	16 (100)	36 (97)
16	Ile	14 (93)	6 (100)	16 (100)	36 (97)
16	Val	1[a] (7)	0 (0)	0 (0)	1 (3)
74	Ala	8 (53)	5 (83)	11 (69)	24 (65)
74	Thr	7 (47)	1 (17)	5 (31)	13 (35)
168	Leu	1[a] (7)	0 (0)	0 (0)	1 (3)
168	Phe	14 (93)	6 (100)	16 (100)	36 (97)

[a] Strain from an identical patient (KMT-45).

Polymorphism of *H. pylori* α-CA and Gastric Mucosal Lesions. Table 1 shows the prevalence of amino acid substitutions at the six residues described above among the 37 *H. pylori* strains from patients with a variety of gastric mucosal lesions.

There were some variations in the prevalence of polymorphism at ^{10}A/V and ^{74}A/T. However, there was no statistical significance of differences in their prevalence among the three types of gastric mucosal lesions, indicating thus a normal polymorphism. All strains, except KMT-45, showed ^{12}Ala, ^{13}Thr, ^{16}Ile, and ^{168}Phe (see KMT-12 in Figure 1). At these residue numbers, KMT-45 used the same amino acids as the clone reported by Chirica et al.[27]

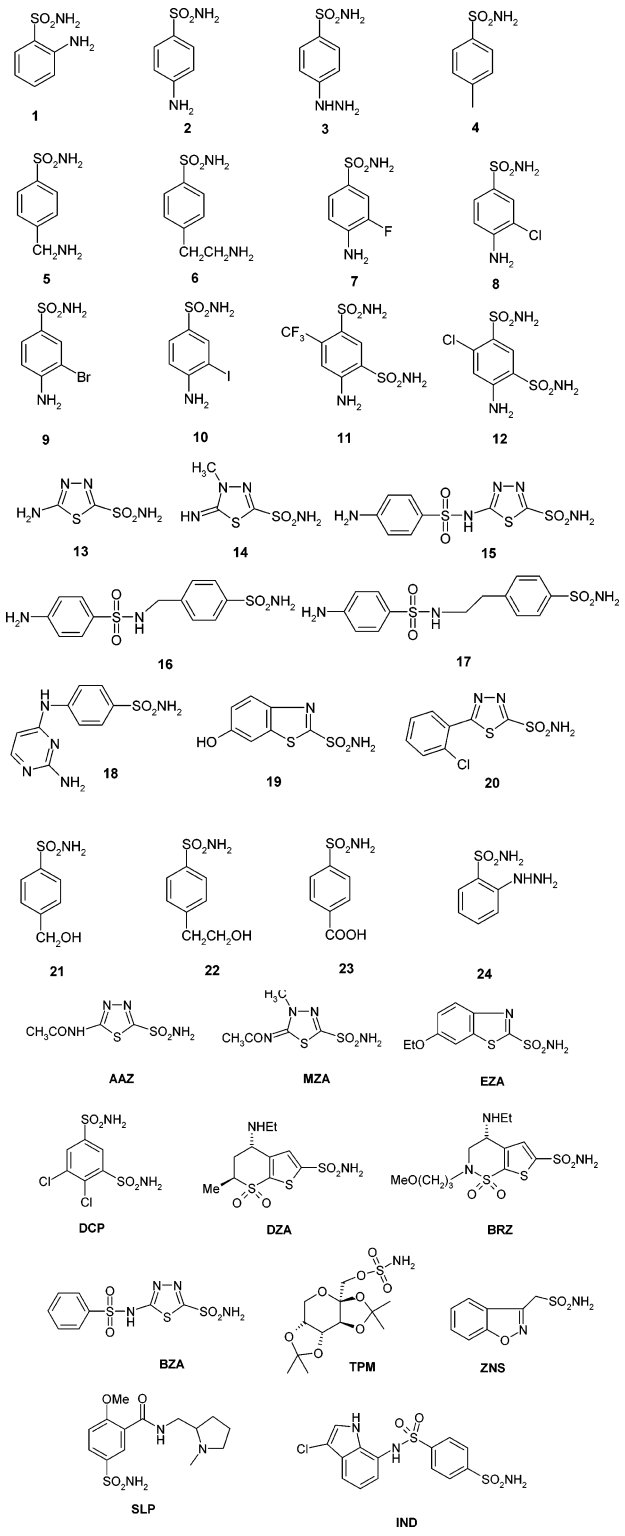
Recombinant hpCA Preparation. Chirica et al.[27] reported that the full-length gene product of *H. pylori* α-CA is possibly toxic to *Escherichia coli* host cell BL21(DE3). In the present study, we tried to produce both full-length and N-terminal truncated recombinant proteins for the hpCA by using *E. coli* JM109. As previously reported,[27] these bacteria failed to produce the full-length hpCA but successfully produced the N-terminal truncated one. Accordingly, we employed a mature form of hpCA lacking a putative N-terminal signal sequence of 18 amino acid residues for the inhibition study. It is of note that the hpCA clone encoding the recombinant protein inherits the identical polymorphism types as KMT-12 clone does (Figure 1).

Chemistry and CA Inhibition. A large number of derivatives was investigated for the inhibition of the bacterial enzymes hpCA. Simple aromatic and heteroaromatic sulfonamides of types **1**−**24** (Chart 1) are among them. Derivatives AAZ-IND (Chart 1) are clinically used drugs: acetazolamide AAZ, methazolamide MZA, ethoxzolamide EZA and dichlorophenamide DCP, are the classical, systemically acting CA inhibitors (CAIs).[1−3] Dorzolamide DZA and brinzolamide BRZ are topically acting antiglaucoma agents,[1,4] benzolamide BZA is an orphan drug belonging to this class of pharmacological agents, whereas topiramate TPM and zonisamide ZNS are widely used antiepileptic drugs.[11b,32] Sulpiride SLP[33] and indisulam IND[34] were recently shown by this group to belong to this class of pharmacological agents. Compounds **1**, **2**, **4**−**6**, **11**, **12**, **18**−**20**, **23**, and AAZ-SLP are commercially available, whereas **3**, **7**−**10**,[35] **13**−**17**,[36,37] **21**, **22**,[38] and **24**,[39] were prepared as reported earlier by this group.

Since *H. pylori* has both an outer as well as an inner membrane,[24,25] and as it is still not very clear whether the main target of the sulfonamide drugs inhibiting its growth is the α-class enzyme we have cloned here (hpCA), the *β*-CA found in the cytoplasm,[26] or both of them, we have also decided to prepare sulfonamides incorporating lipophilic tails in their molecules, which may show a facilitated membrane permeability. We have obtained 12 such derivatives using benzenesulfonamide/1,3,4-thiadiazole-2-sulfonamide scaffolds to which the highly lipophilic 4-*tert*-butylphenylcarboxamido or 4-*tert*-butylphenylsulfonamido tails have been attached by the procedures previously developed by our group.[37] Thus, reaction of amino-containing sulfonamides with 4-*tert*-butylbenzoyl choride A or 4-*tert*-butylbenzenesulfonyl chloride B afforded the new compounds **25**−**36** (Scheme 1).

Inhibition data of these sulfonamides/sulfamates against the host isozymes hCA I and hCA II (highly abundant in the blood and gastrointestinal tract)[1−3] and the bacterial enzyme hpCA are shown in Table 2.[40,41]

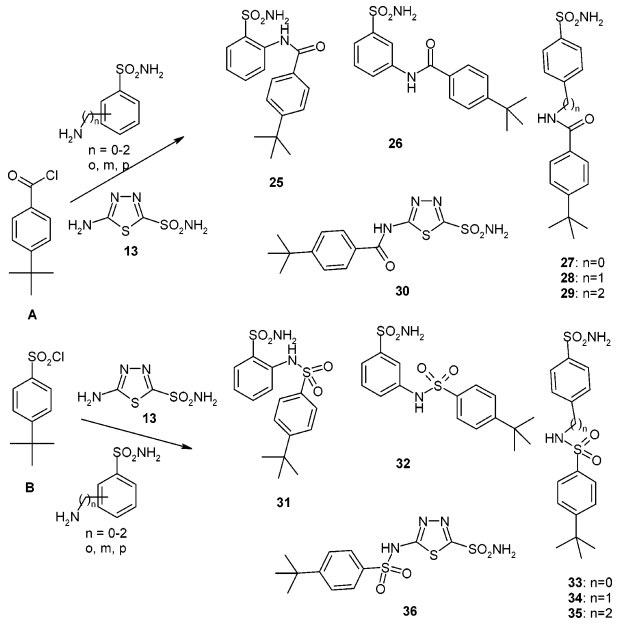
Chart 1



Discussion

Sequence and Polymorphism of *H. pylori* α-CA in Gastric Mucosal Lesions. Two different length polypeptides (202 and 247 residues) were previously reported for hpCA.[27,29−31] In the present study, DNA sequences of 37 independent *H. pylori* strains encoding an open reading frame with 247 amino acids have been analyzed, indicating that this is the exact wild-type protein length. During the screening analysis for the hpCA sequence in a panel of *H. pylori* strains, several new amino acid substitutions were found. These findings prompted us to study a possible relevance of the polymorphisms in the hpCA amino acid sequence, correlating them with gastric mucosal lesion types in which each strain might be involved, since the hpCA (as well as the *β*-class CA found in the cytoplasm of this pathogen) were suggested to function in urea and bicarbonate metabolism and acid resistance/acclimation of these bacteria.[26] However, the sequence analyses in a panel of strains from

Scheme 1



patients with gastritis, gastric ulcer, and gastric cancer failed to show significant relevance of any polymorphism with gastric mucosal lesion types (Table 1). Thus, hpCAs from patients with different types of gastric lesions are all similar, pointing out that drugs able to inhibit them might be used in the management of gastritis/gastric ulcers as well as gastric tumors.

Interestingly, all strains except one (KMT-45, from a patient with gastritis) showed the following amino acid substitutions: ^{12}Ala, ^{13}Thr, ^{16}Ile, and ^{168}Phe. Although ^{16}Ile and ^{168}Phe were previously reported in other clones, ^{12}Ala and ^{13}Thr were newly found in our study. The present study employed *H. pylori* strains isolated from Japanese patients, whereas other sequences were previously obtained from the strain 26695, which was isolated from a patient with gastritis in the United Kingdom,[31] or from the strain J99, which was isolated from a patient with duodenal ulcer in the United States.[29] Accordingly, *H. pylori* strains with ^{12}Ala and ^{13}Thr in the α-CA sequence could be hallmarks for specific variants of the pathogen found in Japan. Probably the KMT-45 strain obtained in the present study might be of foreign origin and infected a Japanese patient.

Chemistry and CA Inhibition. Sulfonamides and sulfamates are well-known for their high affinity for many α-CA isozymes, acting as potent inhibitors with clinical applications as antiglaucoma, diuretic, antiobesity, or antitumor drugs.[1−5,28] Various isoforms are responsible for specific physiological functions, and drugs with such a diversity of actions target in fact quite different isozymes.[1−5,28] In all of them, the sulfonamide/sulfamate drug binds in deprotonated form to the catalytically critical Zn(II) ion, also participating in extensive hydrogen-bond and van der Waals interactions with amino acid residues both in the hydrophobic and hydrophilic halves of the enzyme active site, as shown by X-ray crystallographic work of enzyme−inhibitor complexes.[10,11,13,32]

In the early 1970s it was reported in the literature that acetazolamide, AAZ, the CA inhibitor par excellence, is also effective in the therapy of gastric and duodenal ulcers.[42] The antiulcer effects of this potent CAI (developed in the 1950s as the first nonmercurial diuretic)[43] was assigned as being due to the inhibition of CA isoforms present in gastric mucosa, mainly CA I and II, which were considered to be involved in gastric acid secretion due to H^+ ions generated by hydration of carbon dioxide.[42,43] Interestingly, although the treatment of ulcers with CAIs has not been widely used except by Puscas[42] (also because the H2-receptor antagonists and the PPIs were discovered in the late 1970s and 1980s, respectively), this approach was quite successful, since the healing rate after 30 days of AAZ was 94% (as compared to 48% for antacid-treated patients), and the relapse after 2 years was only 6.2% after AAZ treatment as compared to a relapse of 34% for the antacid-treated patients.[42] In light of the recent findings of Sachs' group[24,25] that hpCA is essential for the life cycle of *H. pylori* (its absence in knockout bacteria or due to inhibition with AAZ leading to a 3 log unit decrease of the pathogen survival in acid), and considering that AAZ is probably a potent hpCA inhibitor (as mentioned by Lindskog's group[27] but without any quantitative inhibition data), we may reinterpret Puscas' data[42] as another proof-of-concept "experiment" that hpCA inhibitors can successfully be used for

Table 2: Inhibition of *H. pylori* Carbonic Anhydrase and of the Human Isoforms hCA I and hCA II, with Compounds **1**−**35** and the 11 Clinically Used Sulfonamides/Sulfamates **AAZ**−**IND**

inhibitor	K_I[a] (nM) hCA I[b]	hCA II[b]	hpCA[c]	selectivity ratio, hCA II/hpCA
1	45 400	295	426	0.69
2	25 000	240	454	0.52
3	28 000	300	316	0.94
4	78 500	320	450	0.71
5	25 000	170	873	0.19
6	21 000	160	1150	0.14
7	8300	60	1230	0.04
8	9800	110	378	0.29
9	6500	40	452	0.08
10	6000	70	510	0.13
11	5800	63	412	0.15
12	8400	75	49	1.53
13	8600	60	323	0.18
14	9300	19	549	0.03
15	6	2	268	0.007
16	164	46	131	0.35
17	185	50	114	0.43
18	109	33	84	0.39
19	95	30	207	0.14
20	690	12	105	0.11
21	55	80	876	0.09
22	21 000	125	1134	0.11
23	23 000	133	1052	0.12
24	24 000	125	541	0.23
AAZ	250	12	21	0.57
MZA	50	14	225	0.06
EZA	25	8	193	0.04
DCP	1200	38	378	0.10
DZA	50 000	9	4360	0.002
BRZ	45 000	3	210	0.01
BZA	15	9	315	0.02
TPM	250	10	172	0.05
ZNS	56	35	231	0.15
SLP	1200	40	204	0.19
IND	31	15	413	0.03
25	12 300	241	539	0.44
26	10 750	210	316	0.66
27	14 250	133	79	1.68
28	13 270	127	62	2.04
29	12 450	123	51	2.41
30	541	18	13	1.38
31	14 700	354	640	0.55
32	9620	203	318	0.63
33	13 000	119	60	1.98
34	12 150	104	31	3.35
35	12 045	94	27	3.48
36	338	15	12	1.25

[a] Errors in the range of 5−10% of the shown data, from three different assays. [b] Human recombinant isozymes, stopped-flow CO_2 hydrase assay method.[41] [c] Recombinant *H. pylori* enzyme lacking the first N-terminal signal sequence of 18 amino acid residues, stopped-flow CO_2 hydrase assay method.[41]

the management of gastric diseases. Thus, we decided to investigate a library of sulfonamides/sulfamates (of types **1**−**24** and AAZ-IND) for their interaction with hpCA, to detect potent inhibitors with potential use as gastric drugs.

H. pylori has two CAs (the periplasmic hpCA belonging to the α-class, and a cytoplasmic *β*-CA hypothesized to be also involved in the bicarbonate/urea metabolism and survival in acid media).[26] A drug targeting these enzymes should cross easily one (for the hpCA targeting) or two membranes (for the *β*-CA targeting) in order to inhibit them. Thus, we decided to prepare sulfonamides with enhanced lipophilicity and thus a facilitated access through biological membranes. A series of such derivatives were obtained by using benzenesulfonamide/1,3,4-thiadiazole-2-sulfonamide scaffolds to which the highly lipophilic 4-*tert*-butylphenylcarboxamido or 4-*tert*-butylphenylsulfonamido tails have been attached by the procedures previously developed by this group.[37] Reaction of amino-containing benzenesulfonamides or 5-amino-1,3,4-thiadiazole-2-sulfonamide with 4-*tert*-butylbenzoyl choride A or 4-*tert*-butylbenzenesulfonyl chloride B in the presence of triethylamine or in Schotten−Baumann conditions afforded the new compounds **25**−**36** (Scheme 1). Compounds **25**−**36** reported here were characterized by routine spectroscopic and analytic procedures that confirmed their structures (see Experimental Section for details).

The hpCA preparations obtained in this study by means of a GST fusion protein technique, previously reported for other α-CAs by our group,[9,11,12] showed a catalytic activity similar to the enzyme reported earlier by Lindskog's group[27] (for the CO_2 hydration reaction), with a k_{cat} of 2.5×10^5 s^{-1} and k_{cat}/K_M of 1.47×10^7 M^{-1} s^{-1} at 25 °C and pH of 8.9. Thus, hpCA is rather similar to hCA I from the catalytic viewpoint (k_{cat} of 2.0×10^5 and k_{cat}/K_M of 5×10^7 under the same conditions),[9a] as already noted by Chirica et al.[27] However, in contrast to the previous preparation method,[27] the GST fusion protein technique allows the facile preparation of rather high amounts of recombinant hpCA (see Experimental Section for details), needed for the inhibitor screening studies.

Data of Table 2 show that all 47 compounds investigated here act as inhibitors of the bacterial enzyme hpCA, at the same time being inhibitory against the host α-CA isoforms hCA I and II, but with a quite different behavior against the three targets (data for hCA I and II of compounds **1**−**24** and AAZ-IND have previously been published[4,11−13] but are included for discussing the selectivity issue of these CAIs for the bacterial versus the host enzymes; see later in the text). The hCA I ZNS data, are new, together with the hpCA inhibition data of all 47 derivatives. The hCA I and II data of derivatives **25**−**36** are also reported here for the first time. The following SAR can be drawn from data of Table 2 for the library of investigated sulfonamides/sulfamates: (i) A group of derivatives, including **5**−**7**, **21**−**23**, and DZA, acted as very weak hpCA inhibitors, with inhibition constants in the range 873−4360 nM. Except for the heterocyclic sulfonamide DZA, all these compounds are benzenesulfonamide derivatives possessing moieties substituting the benzene ring in the para position with respect to the sulfamoyl group, of the aminomethyl/ethyl, hydroxymethyl/ethyl, or carboxy type (**7** is the fluorinated derivative of sulfanilamide). It may also be noted that all these derivatives act as much better hCA II and as weaker hCA I inhibitors, as compared to their activity on hpCA. (ii) Derivatives **1**, **2**, **4**, **9**−**11**, **14**, **24**, IND, **25**, and **31** were better hpCA inhibitors as compared to the previously mentioned sulfonamides, with inhibition constants in the range 413−640 nM. From the structural point of view, these sulfonamides belong to a rather heterogeneous group of derivatives, being either orthanilamides (**1**, **24**, **25**, and **31**), 4-substituted benzenesulfonamides (**2**, **4**, IND), halogenated sulfanilamides (**9** and **10**), or the heterocyclic compound **14** and the benzene-1,3-disulfonamide derivative **11**. As the previously discussed sulfonamides, also these derivatives are much better hCA II inhibitors and much weaker hCA I inhibitors, as compared to their action on the bacterial enzyme. (iii) A group of 18 derivatives, including **3**, **8**, **13**, **15**−**17**, **19**, **20**, MZA, EZA, DCP, BRZ, BZA, TPM, ZNS, SLP, **26**, and **32** showed medium potency as hpCA inhibitors, with K_Is in the range of 105−378 nM. Again a rather large structural heterogeneity was noted, with some derivatives being (3),4-(di)substituted benzenesulfonamides/halogenated sulfanilamides (**3**, **8**, **16**, **17**, DCP and SLP) or substituted metanilamides (**26** and **32**) but most of them belonging to the heteroaromatic class of sulfonamides, mainly 1,3,4-thiadiazole-2-sulfonamide (**20**, MZA, BZA) and benzothiazole-2-sulfonamide derivatives (**19**, EZA). The clinically used antiglaucoma heterocyclic sulfonamide BRZ, the antiepileptic sulfamate TPM, and the aliphatic sulfonamide ZNS also belong to this class of medium-potency hpCA inhibitors. The tremendous difference of activity between DZA and BRZ should be noted, although the two compounds are structurally similar. Thus, DZA is approximately 21 times less effective as a hpCA inhibitor as compared to BRZ (these two compounds are also very weak hCA I inhibitors but very potent hCA II inhibitors). On the other hand, all these sulfonamides/sulfamates act as very potent hCA II inhibitors (Table 2), whereas their activity on hCA I is much more variable, as some of them are strong inhibitors (**15**, **21**, MZA, EZA, BZA, ZNS, IND), others are medium-potency inhibitors, whereas others, as mentioned above, are quite weak hCA I inhibitors (**3**, **8**, **13**, DZA, BRZ, **26**, and **32**). (iv) A last group of 11 derivatives (**12**, **18**, AAZ, **27**−**30**, and **33**−**36**) showed potent hpCA inhibitory activity, with inhibition constants in the range of 12−84 nM. Except for the benzene-1,3-disulfonamide **12**, the 4-aminopyrimidinylsulfanilamide derivative **18**, and acetazolamide AAZ (the CA inhibitor par excellence), these strong inhibitors were newly designed in the present study and incorporate the 4-*tert*-butylphenylcarboxamido/-sulfonamido moieties (derivatives **27**−**30** and **33**−**36**). Several findings should be noted here. A large difference of activity against hpCA has been found for the two strucurally related benzene-1,3-disulfonamides **11** and **12**, with the last one being 8.40 times better as an inhibitor as compared to **11**. Thus, the bulkier trifluoromethyl group in the 6 position of the benzene ring is detrimental to activity on hpCA as compared to a chlorine atom in the same position. Derivatization of the sulfanilamide **2**, homosulfanilamide **5**, or 4-aminoethylbenzenesulfonamide **6** leads to molecules (which are all weak hpCA inhibitors) with bulky moieties, such as those present in derivatives **16**−**18**, **27**−**29**, and **33**−**35**, and leads to an important increase (around 4.5−45 times) of the hpCA inhibitory activity (generally paralleled by the increase of hCA I and hCA II inhibitory activities too). However, the best inhibitors of the bacterial enzyme were the acetazolamide-like derivatives **30** and **36**, incorporating the lipophilic 4-*tert*-butylphenylcarboxamido/-sulfonamido moieties (K_I 12−13 nM), together with acetazolamide AAZ itself, a compound known for its usefulness in treating *H. pylori*-mediated diseases.[42] We also confirm the strong inhibitory activity of AAZ against hpCA, as mentioned by Chirica et al.[27] (but with no quantitative data published in their work), which possesses a K_I of 21 nM. It should be observed that the very strong hpCA inhibitors **30** and **36** were also

obtained from a rather inefficient lead (the deprotected precursor of acetazolamide **13**, which has a K_I of 323 nM), with an enhancement of 15−27 times the inhibitory activity by derivatization/acetylation (Table 2). It should be also noted that generally the 4-*tert*-butylphenylcarboxamido derivatives **27**−**30** were slightly less efficient hpCA inhibitors as compared to the corresponding 4-*tert*-butylphenylsulfonamido derivatives **33**−**36**. (v) A main issue regarding the CAIs is their selectivity for the target isozyme, considering the fact that the ubiquitous human isoform hCA II (which is also a target for many types of CAI-based drugs)[1−5] has a high affinity for sulfonamides/sulfamates.[1−5] Indeed, as observed from data of Table 2, most of the investigated compounds from this study are better hCA II than hpCA inhibitors, with selectivity ratios below unity (e.g., compounds **1**−**11**, **12**−**24**, AAZ-IND, **25**, **26**, **31**, and **32** have selectivity ratios in the range of 0.002−0.94). However, some of the investigated compounds, such as **12, 27**−**30**, and **33**−**36**, possess selectivity ratios in the range of 1.38−3.48, being thus better inhibitors of the bacterial over the host enzyme. These selectivity ratios are not very high, but they are much better than those of acetazolamide (selectivity ratio of 0.57), the compound known to be effective in humans in treating *H. pylori*-mediated diseases.[25,42] Thus, **35**, with a K_I similar to AAZ but a 3.48 better hpCA inhibitor than a hCA II inhibitor, might show some advantages as compared to the clinically used compound in the management of *H. pylori* infection. Work is in progress in our laboratories to detect even more selective and potent hpCA inhibitors belonging to other classes of compounds.

Conclusions

We have cloned and purified *H. pylori* CA from patients with different gastric mucosal lesions. The enzymes from gastritis, gastric ulcer, and gastric cancer patients were identical, possessing 247 amino acid residues. Several new polymorphisms have been identified in this protein in Japanese patients, such as for example ^{12}Ala, ^{13}Thr, ^{16}Ile, and ^{168}Phe. Strains with ^{12}Ala and ^{13}Thr substitutions in the hpCA sequence seem to be hallmarks for specific variants of the pathogen found in Japan. A library of sulfonamides/sulfamates has been investigated for the inhibition of this enzyme, whereas new lipophilic derivatives have been obtained by attaching 4-*tert*-butylphenylcarboxamido/sulfonamido tails to benzenesulfonamide/1,3,4-thiadiazole-2-sulfonamide scaffolds. All types of activity for inhibition of the bacterial enzyme have been detected. Dorzolamide and simple 4-substituted benzenesulfonamides were weak hpCA inhibitors (K_I 873−4360 nM). Sulfanilamide, orthanilamide, some of their derivatives and indisulam showed better activity (K_I 413−640 nM), whereas most of the clinically used CA inhibitors, such as methazolamide, ethoxzolamide, dichlorophenamide, brinzolamide, topiramate, zonisamide, etc., acted as medium-potency hpCA inhibitors (K_I 105−378 nM). Some potent hpCA inhibitors were detected too (K_I 12−84 nM) among acetazolamide, 4-amino-6-chloro-1,3-benzenedisulfonamide, and some of the compounds incorporating 4-*tert*-butylphenylcarboxamido/sulfonamido tails, newly designed during this work. Most of the investigated derivatives acted as better hCA II than hpCA inhibitors. However, some of the newly prepared derivatives had selectivity ratios for inhibiting hpCA over hCA II in the range of 1.25−3.48, showing thus some selectivity for inhibiting the bacterial enzyme. Since hpCA is essential for the survival of the pathogen in acid, its inhibition by compounds as those reported here might be used as a new pharmacologic tool in the management of drug-resistant *H. pylori*.

Experimental Section

Chemistry. Melting points were recorded with a heating plate microscope and are not corrected. IR spectra were obtained in KBr pellets with a Carl Zeiss UR 20 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Gemini 300BB apparatus operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, in DMSO-d_6 as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. Attributions were done by means of chemical shifts, peak integration, COSY (^{1}H−^{1}H), HETCOR (^{1}H−^{13}C), attached proton test (APT), model spectra, and selective deuteration. Elemental analyses were done by combustion for C, H, and N with an automated Carlo Erba analyzer and were ±0.4% of the theoretical values. All reactions were monitored by thin-layer chromatography (TLC) on 0.25-mm precoated silica gel plates (E. Merck). Compounds **1**, **2**, **4**−**6**, **11**, **12**, **18**−**20**, **23**, and AAZ-SLP are commercially available from Sigma−Aldrich, Merck, Alcon, DaiNippon, or Johnson & Johnson, whereas **3**, **7**−**10**,[35] **13**−**17**,[36,37] **21**, **22**,[38] and **24**[39] were prepared as reported earlier by this group. Acyl chloride A, sulfonyl chloride B, solvents, and other organic/inorganic reagents were commercially available, from Sigma−Aldrich (Milan, Italy). Sulfonamides **25**−**36** are new and were prepared as described below.

General Procedure for the Synthesis of Sulfonamides 25−36: Method A (Schotten−Baumann synthesis). Five millimoles of aminosulfonamide to be derivatized (such as, for instance, 5-amino-1,3,4-thiadiazole-2-sulfonamide **13**) was dissolved in 15 mL of an aqueous 2.5 M solution of NaOH and cooled to 0−5 °C in a salt-ice bath. Sulfonyl/acyl chloride A/B (5 mmol) was added in small portions concomitantly with 10 mL of a cold 2 M NaOH solution, with the temperature maintained below 10 °C. The reaction mixture was stirred at room temperature for 5−10 h (TLC control), then the pH was adjusted to 2 with 5 N HCl, and the precipitated sulfonamides were filtered and recrystallized from aqueous ethanol. Yields were in the range 25−79%.

Method B. The aminosulfonamide **2**, **5**, **6**, **13**, or **1** (1 g) was dissolved in *N*,*N*-dimethylacetamide (10 mL), and 1 equiv of sulfonyl/acyl chloride **A**/**B** was added in small portions, together with 1 equiv of sodium bicarbonate or triethylamine. The reaction mixture was left on ice under stirring. After 60 min, 50 mL of water was added to the reaction mixture, which was then extracted three times with ethyl acetate (25 mL). The organic fractions were collected and extracted three times with a 1 N hydrochloric acid aqueous solution (15 mL). The organic phase was dried over magnesium sulfate. After charcoal treatment, the organic phase was dried under depression. The final compounds were recrystallized as mentioned above. The purity of the final compound was verified by TLC (MeOH/$CHCl_3$ 3/7).

2-(4-*tert*-Butylphenylcarboxamido)benzenesulfonamide, 25 (Method A): white crystals, mp 129−31 °C (EtOH); IR (KBr) (cm^{-1}) 1160 (SO_2^{sym}), 1325 (SO_2^{as}), 1585 (amide II), 1650 (amide I); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.98 (s, 9H, t-Bu), 7.26 (s, 2H, SO_2NH_2), 7.54−7.80 (m, 4H, ArH), 7.76 (d, 2H, AA′BB′, 8.8), 7.89 (d, 2H, AA′BB′, 8.8), 10.43 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.20 (Me); 61.37 (C from t-Bu); 126.61 (C2/C3 of 1,4-phenylene), 127.39 (C of 1,2-phenylene), 128.35 (C3/C2 of 1,4-phenylene), 129.96 (C of 1,2-phenylene), 137.55 (C2 of 1,2-phenylene); 139.49 (C1/C4 of 1,4-phenylene), 140.76 (C1 of 1,2-phenylene), 142.25 (C4/C1 of 1,4-phenylene), 160.13 (CONH); Anal. ($C_{17}H_{20}N_2O_3S$) C, H, N.

3-(4-*tert*-Butylphenylcarboxamido)benzenesulfonamide, 26 (Method B):, white crystals, mp 219−20 °C (MeOH); IR (KBr) (cm^{-1}) 1165 (SO_2^{sym}), 1330 (SO_2^{as}), 1585 (amide II), 1645 (amide I); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.90 (s, 9H, t-Bu), 7.37 (s, 2H, SO_2NH_2), 7.26−7.61 (m, 3H, ArH), 7.74 (d, 2H, AA′BB′, 8.8), 7.83 (d, 2H, AA′BB′, 8.8), 7.92 (s, 1H, ArH), 10.40 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.39 (Me); 61.40 (C from t-Bu); 126.73 (C2/C3 of 1,4-phenylene), 127.87 (C of 1,3-phenylene), 128.31 (C3/C2 of 1,4-phenylene), 130.63 (C of 1,3-phenylene), 135.70 (C of 1,3-phenylene), 138.39 (C3 of 1,3-phenylene), 139.64 (C1/C4 of 1,4-phenylene), 142.33 (C1 of

1,3-phenylene), 142.56 (C4/C1 of 1,4-phenylene), 161.59 (CONH); Anal. ($C_{17}H_{20}N_2O_3S$) C, H, N.

4-(4-*tert*-Butylphenylcarboxamido)benzenesulfonamide, 27 (Method B): white crystals, mp 250−1 °C (MeOH); IR (KBr) (cm^{-1}) 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 1585 (amide II), 1650 (amide I); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.93 (s, 9H, t-Bu), 7.30 (s, 2H, SO_2NH_2), 7.73 (d, 2H, AA′BB′, 8.8), 7.82 (d, 2H, AA′BB′, 8.9), 7.85 (d, 2H, AA′BB′, 8.8), 7.92 (d, 2H, AA′BB′, 8.9), 10.51 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.25 (Me); 61.54 (C from *t*-Bu); 126.67 and 126.74 (C2/C3 of Ph), 128.26 and 128.35 (C3/C2 of Ph), 139.49 and 139.80 (C1/C4 of Ph), 141.80 and 142.25 (C4/C1 of Ph), 160.28 (CONH); Anal. ($C_{17}H_{20}N_2O_3S$) C, H, N.

4-(4-*tert*-Butylphenylcarboxamidomethyl)benzenesulfonamide, 28 (Method A): white crystals, mp 220−1 °C (EtOH); IR (KBr) (cm^{-1}) 1160 (SO_2^{sym}), 1310 (SO_2^{as}), 1550 (amide II), 1620 (amide I); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.92 (s, 9H, t-Bu), 4.51 (d, 1H, 6.0), 7.33 (s, 2H, SO_2NH_2), 7.49 (d, 2H, AA′BB′, 8.3), 7.73 (d, 2H, AA′BB′, 8.8), 7.80 (d, 2H, AA′BB′, 8.3), 7.85 (d, 2H, AA′BB′, 8.8), 9.15 (t, 1H, NHCO, 5.8); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.38 (Me), 42.23 (CH_2), 61.75 (C from *t*-Bu), 125.82 and 126.41 (C2/C3 of Ph), 127.59 and 128.66 (C3/C2 of Ph), 139.60 and 141.35 (C1/C4 of Ph), 143.61 and 144.72 (C4/C1 of Ph), 161.32 (CONH); Anal. ($C_{18}H_{22}N_2O_3S$) C, H, N.

4-(4-*tert*-Butylphenylcarboxamidoethyl)benzenesulfonamide, 29 (Method B): white crystals, mp 233−4 °C (MeOH); IR (KBr) (cm^{-1}) 1160 (SO_2^{sym}), 1330 (SO_2^{as}), 1550 (amide II), 1610 (amide I);^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.93 (s, 9H, t-Bu), 2.91 (t, 2H, 7.2), 3.49 (q, 2H, 6.4), 7.31 (s, 2H, SO_2NH_2), 7.43 (d, 2H, AA′BB′, 8.3), 7.68 (d, 2H, AA′BB′, 8.8), 7.75 (d, 2H, AA′BB′, 8.1), 7.81 (d, 2H, AA′BB′, 8.8), 8.62 (t, 1H, NHCO, 5.7); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.40 (Me), 34.85 (CH_2-Ph), 40.22 (N-CH_2), 61.60 (C from *t*-Bu), 127.92 and 128.50 (C2/C3 of Ph), 129.20 and 131.24 (C3/C2 of Ph), 139.99 and 141.24 (C1/C4 of Ph), 143.71 and 144.65 (C4/C1 of Ph), 161.16 (CONH); Anal. ($C_{19}H_{24}N_2O_3S$) C, H, N.

5-(4-*tert*-Butylphenylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide, 30 (Method A): white crystals, mp 270−2 °C (EtOH); IR (KBr) (cm^{-1}) 1170 (SO_2^{sym}), 1310 (SO_2^{as}), 1540 (amide II), 1650 (amide I); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.89 (s, 9H, t-Bu), 7.71 (d, 2H, AA′BB′, 8.7), 7.81 (d, 2H, AA′BB′, 8.7), 8.38 (s, 2H, SO_2NH_2), 13.62 (br s, 1H, NHCO); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.57 (Me), 61.31 (C from t-Bu), 128.33 (C2/C3 of Ph), 131.17 (C3/C2 of Ph), 141.56 (C1/C4 of Ph), 144.28 (C4/C1 of Ph), 160.13 (CONH), 162.01 (C-thiadiazole), 164.71 (C-thiadiazole); Anal. ($C_{13}H_{16}N_4O_3S_2$) C, H, N.

2-(4-*tert*-Butylphenylsulfonamido)benzenesulfonamide, 31 (Method A): white crystals, mp 164−6 °C (EtOH); IR (KBr) (cm^{-1}) 1135 and 1160 (SO_2^{sym}), 1325 and 1370 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.90 (s, 9H, t-Bu), 7.35 (s, 2H, SO_2NH_2), 7.51−7.78 (m, 4H, ArH), 7.78 (d, 2H, AA′BB′, 8.9), 7.90 (d, 2H, AA′BB′, 8.9), 11.69 (s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.35 (Me); 61.27 (C from t-Bu); 126.12 (C2/C3 of 1,4-phenylene), 127.03 (C of 1,2-phenylene), 128.30 (C3/C2 of 1,4-phenylene), 129.87 (C of 1,2-phenylene), 137.24 (C2 of 1,2-phenylene); 139.58 (C1/C4 of 1,4-phenylene), 140.61 (C1 of 1,2-phenylene), 142.05 (C4/C1 of 1,4-phenylene); Anal. ($C_{16}H_{20}N_2O_4S_2$) C, H, N.

3-(4-*tert*-Butylphenylsulfonamido)benzenesulfonamide, 32 (Method A): white crystals, mp 254−6 °C (MeOH−water 1:1); IR (KBr) (cm^{-1}) 1135 and 1165 (SO_2^{sym}), 1330 and 1350 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.92 (s, 9H, t-Bu), 7.30 (s, 2H, SO_2NH_2), 7.26−7.61 (m, 3H, ArH), 7.75 (d, 2H, AA′BB′, 8.9), 7.873 (d, 2H, AA′BB′, 8.9), 7.90 (s, 1H, ArH), 11.23 (s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.30 (Me); 61.51 (C from t-Bu); 126.73 (C2/C3 of 1,4-phenylene), 127.34 (C of 1,3-phenylene), 128.93 (C3/C2 of 1,4-phenylene), 130.15 (C of 1,3-phenylene), 135.47 (C of 1,3-phenylene), 138.42 (C3 of 1,3-phenylene), 139.20 (C1/C4 of 1,4-phenylene), 142.13 (C1 of 1,3-phenylene), 142.75 (C4/C1 of 1,4-phenylene); Anal. ($C_{16}H_{20}N_2O_4S_2$) C, H, N.

4-(4-*tert*-Butylphenylsulfonamido)benzenesulfonamide, 33 (Method A): white crystals, mp 224−6 °C (MeOH−water 2:1); IR (KBr) (cm^{-1}) 1130 and 1160 (SO_2^{sym}), 1330 and 1335 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.97 (s, 9H, t-Bu), 7.34 (s, 2H, SO_2NH_2), 7.73 (d, 2H, AA′BB′, 8.8), 7.84 (d, 2H, AA′BB′, 8.9), 7.87 (d, 2H, AA′BB′, 8.8), 7.90 (d, 2H, AA′BB′, 8.9), 11.28 (s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.20 (Me); 61.47 (C from t-Bu); 126.67 and 126.80 (C2/C3 of Ph), 128.32 and 128.54 (C3/C2 of Ph), 139.13 and 139.68 (C1/C4 of Ph), 141.71 and 142.65 (C4/C1 of Ph); Anal. ($C_{16}H_{20}N_2O_4S_2$) C, H, N.

4-(4-*tert*-Butylphenylsulfonamidomethyl)benzenesulfonamide, 34 (Method A): white crystals, mp 245−6 °C (MeOH); IR (KBr) (cm^{-1}) 1145 and 1160 (SO_2^{sym}), 1320 and 1355 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.96 (s, 9H, t-Bu), 4.50 (d, 1H, 6.0), 7.34 (s, 2H, SO_2NH_2), 7.50 (d, 2H, AA′BB′, 8.3), 7.71 (d, 2H, AA′BB′, 8.8), 7.84 (d, 2H, AA′BB′, 8.3), 7.89 (d, 2H, AA′BB′, 8.8), 10.27 (t, 1H, SO_2NH, 6.1); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.08 (Me), 42.27 (CH_2), 61.50 (C from t-Bu), 126.09 and 126.41 (C2/C3 of Ph), 127.83 and 128.69 (C3/C2 of Ph), 139.76 and 140.97 (C1/C4 of Ph), 143.15 and 143.90 (C4/C1 of Ph); Anal. ($C_{17}H_{22}N_2O_4S_2$) C, H, N.

4-(4-*tert*-Butylphenylsulfonamidoethyl)benzenesulfonamide, 35 (Method B): white crystals, mp 218−20 °C (EtOH); IR (KBr) (cm^{-1}) 1145 and 1160 (SO_2^{sym}), 1330 and 1360 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.93 (s, 9H, t-Bu), 2.90 (t, 2H, 7.2), 3.52 (q, 2H, 6.4), 7.30 (s, 2H, SO_2NH_2), 7.46 (d, 2H, AA′BB′, 8.3), 7.69 (d, 2H, AA′BB′, 8.8), 7.75 (d, 2H, AA′BB′, 8.1), 7.80 (d, 2H, AA′BB′, 8.8), 10.17 (t, 1H, SO_2NH, 5.9); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.12 (Me), 34.80 (CH_2-Ph), 40.13 (N-CH_2), 61.37 (C from t-Bu), 127.60 and 128.14 (C2/C3 of Ph), 129.17 and 130.85 (C3/C2 of Ph), 138.76 and 140.16 (C1/C4 of Ph), 142.80 and 144.19 (C4/C1 of Ph); Anal. ($C_{18}H_{24}N_2O_4S_2$) C, H, N.

5-(4-*tert*-Butylphenylsulfonamido)-1,3,4-thiadiazole-2-sulfonamide, 36 (Method A): white crystals, mp 254−6 °C (EtOH); IR (KBr) (cm^{-1}) 1135 and 1170 (SO_2^{sym}), 1310 and 1330 (SO_2^{as}); ^{1}H NMR (DMSO-d_6) (δ, ppm; *J*, hertz) 0.95 (s, 9H, t-Bu), 7.70 (d, 2H, AA′BB′, 8.7), 7.86 (d, 2H, AA′BB′, 8.7), 8.49 (s, 2H, SO_2NH_2), 13.87 (br s, 1H, SO_2NH); ^{13}C NMR (DMSO-d_6) (δ, ppm) 13.25 (Me), 61.69 (C from t-Bu), 128.23 (C2/C3 of Ph), 131.55 (C3/C2 of Ph), 141.18 (C1/C4 of Ph), 144.62 (C4/C1 of Ph), 162.53 (C-thiadiazole), 164.90 (C-thiadiazole); Anal. ($C_{12}H_{16}N_4O_4S_3$) C, H, N.

***H. pylori* Strain.** For screening analysis for hpCA, a panel of *H. pylori* strains was obtained upon clinical examinations from patients with a variety of gastric mucosal lesions, including 15 patients with gastritis, 6 patients with gastric ulcer, and 16 patients with gastric cancer. All patients were residents in Japan. *H. pylori* was cultured as previously reported.[44] In brief, gastric biopsy specimens obtained by endoscopy were immediately spread onto M-BHM pylori plates (Nikken Biomedical, Kyoto, Japan). Following incubation under microaerophilic conditions by use of the Campy-Pouch system (Becton Dickinson, Cockeysville, MD), cultured bacteria were confirmed as being *H. pylori* by light microscopy with Gram staining or fluorescence microscopy with diamino-*s*-phenylindole staining as previously reported.[44] DNAs were extracted from the bacteria by using a DNeasy kit (Qiagen, Hilden, Germany) and stored at −20 °C until later use.

DNA Sequencing. A panel of DNA samples from the patients was subjected to PCR amplification and sequencing of full-length DNA coding the hpCA. The GenBank database search identified three DNA clones of hpCA. One clone (HP1186, Accession Number AE000511) was obtained from *H. pylori* strain 26695 by TIGR sequencing center,[30,31] and the other (jhp 1112, Accession Number AE001439) was from the strain J99 by ASTRA research center.[29] The remaining clone was obtained from the same *H. pylori* strain, 26695, as the one used by the TIGR sequence center but showed somewhat different DNA sequence of hpCA.[27] Based on 5′- and 3′-uncoding regions of the hpCA sequence of jhp 1112 clone, in the present study, a primer pair was synthesized for polymerase chain reaction (PCR). The sequences of the primer pairs were as

follows: 5′-TAACTGCGGTCATTATTGATTAAGC-3′ and 5′-TCACAAAATACAAGCGGTTGCAAT-3′. The PCR reaction was hot-started with incubation for 1 min at 94 °C and consisted of 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 90 s at 72 °C. The PCR product was sequenced by use of an ABI PRISM dye termination cycle sequencing kit (Perkin-Elmer, Foster City, CA) and an ABI 370A DNA sequencer (ABI, Foster City, CA).

Preparation of Recombinant Protein. The cDNA fragment encoding the open reading frame of hpCA (based on the DNA sequence of jhp 1112, Accession Number AE001439) was amplified from a *H. pylori* strain isolated from a Japanese patient with gastritis. DNA sequencing showed that the hpCA clone inherited the identical polymorphism types as KMT-12 clone did (Figure 1). Because Chirica et al.[27] reported that the full-length gene product of hpCA is possibly toxic to the *E. coli* host cell BL21(DE3), we set two types of 5′-primers: one primer started from the first methionine and another from glutamic acid at the 19th residue, lacking a putative N-terminal signal sequence 18 amino acids long.[27] Sequences for adopter primers including *Bam*HI and *Eco*RI recognition sequences (underlined in the following sequences, respectively) were as follows: 5′-CGGGATCCATGAAAAAAA-CTTTTTTGATCGCTTTA-3′ for the full-length protein, 5′-CG-GGATCCATGGACACCAAATGGGATTATAAGAATAAAGAA-3′ for the N-terminal truncated protein, and 5′-CGGAATTCT-TAGCGGGTCTCAGCCGAGCTTTTAATGATCAC-3′ for the C-terminal end. The PCR reaction was hot-started with incubation for 5 min at 94 °C and consisted of 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 90 s at 72 °C. The PCR products were cleaved with *Bam*HI and *Eco*RI and then ligated in-frame into the pGEX-4T2 vector (Amersham, Tokyo, Japan). The proper DNA sequences of the hpCA insert included in the vector were reconfirmed by DNA sequencing. The constructs were then transfected into *E. coli* strain JM109 for production of the GST−CA fusion protein as previously reported.[9,11,12] Following induction of the proteins expression by adding 1 mM isopropyl γ-D-thiogalactopyranoside, the bacteria were harvested and sonicated in phosphate-buffered saline (PBS). The sonicated cell extracts were further homogenized twice with a Polytron (Brinkmann) for 30 s each at 4 °C. Centrifugation at 30000*g* for 30 min afforded the supernatant containing the soluble proteins. The obtained supernatants were then applied to prepacked glutathione−Sepharose 4B columns (Amersham). The columns were extensively washed with buffer and then the GST−CA fusion protein was eluted with a buffer consisting of 5 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0. Finally the GST parts of the fusion proteins were cleaved with thrombin. The obtained hpCA recombinant proteins were further purified by sulfonamide affinity chromatography,[40] the amount of enzyme being determined by spectrophotometric measurements and its activity by stopped-flow experiments, with CO_2 as substrate.[41]

CA Inhibition Assay. An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[41] Phenol red (at a concentration of 0.2 mM) has been 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), at 25 °C, following the CA-catalyzed CO_2 hydration reaction for a period of 10−100 s (the uncatalyzed reaction needs around 60−100 s in the assay conditions, whereas the catalyzed ones need around 6−10 s). The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters. For each inhibitor, tested in the concentration range between 0.01 nM and 100 μM, 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 (1 mM) were prepared in distilled−deionized water with 10−20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.01 nM were done thereafter with distilled−deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, to allow for the formation of the E−I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3. The curve-fitting algorithm allowed us to obtain the IC_{50} values (working at the lowest concentration of substrate of 1.7 mM), from which K_I values were calculated by using the Cheng−Prusoff equation.[9−11] The catalytic activity (in the absence of inhibitors) of these enzymes was calculated from Lineweaver−Burk plots, as reported earlier, and represent the mean from at least three different determinations.[9−11] Enzyme concentrations in the assay system were: 9.2 nM for hCA I, 7.6 nM for hCA II, and 10.3 nM for hpCA.

Statistics. Differences in the frequency distribution of amino acid polymorphisms among *H. pylori* strains from patients with a variety of gastric mucosal lesions were assessed by the χ^2 test, and differences with P values of <0.05 were considered significant.

Acknowledgment. This work was supported in part by a grant from the Japanese Ministry of Education, Science, Sports and Culture (9770363) and by an EU grant (to C.T.S. and A.S.) of the 6th framework program (EUROXY project).

Supporting Information Available: Elemental analysis data for compounds **25**−**36**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Supuran, C. T.; Scozzafava, A.; Conway, J. *Carbonic Anhydrase—Its Inhibitors and Activators*; CRC Press: Boca Raton, FL, 2004; pp 1−363.
(2) Sly, W. S.; Hu, P. Y. Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu. Rev. Biochem.* **1995**, *64*, 375−401.
(3) Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199−229.
(4) Supuran, C. T.; Scozzafava, A.; Casini, A. Carbonic anhydrase inhibitors. *Med. Res. Rev.* **2003**, *23*, 146−89.
(5) Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Modulation of carbonic anhydrase activity and its applications in therapy. *Expert Opin. Ther. Pat.* **2004**, *14*, 667−702.
(6) Lehtonen, J.; Shen, B.; Vihinen, M.; Casini, A.; Scozzafava, A.; Supuran, C. T.; Parkkila, A. K.; Saarnio, J.; Kivela, A. J.; Waheed, A.; Sly, W. S.; Parkkila, S. Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family. *J. Biol. Chem.* **2004**, *279*, 2719−27.
(7) Fujikawa-Adachi, K.; Nishimori, I.; Taguchi, T.; Onishi, S. Human mitochondrial carbonic anhydrase VB. cDNA cloning, mRNA expression, subcellular localization, and mapping to chromosome X. *J. Biol. Chem.* **1999**, *274*, 21228−33.
(8) Fujikawa-Adachi, K.; Nishimori, I.; Taguchi, T.; Onishi, S. Human carbonic anhydrase XIV (CA14): cDNA cloning, mRNA expression, and mapping to chromosome 1. *Genomics* **1999**, *61*, 74−81.
(9) (a) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Carbonic anhydrase inhibitors. The mitochondrial isozyme VB as a new target for sulfonamide and sulfamate inhibitors. *J. Med. Chem.* **2005**, *48*, 7860−7866. (b) Vullo, D.; Franchi, M.; Gallori, E.; Antel, J.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of mitochondrial isozyme V with aromatic and heterocyclic sulfonamides. *J. Med. Chem.* **2004**, *47*, 1272−9. (c) Supuran, C. T. Carbonic anhydrase inhibitors in the treatment and prophylaxis of obesity. *Expert Opin. Ther. Pat.* **2003**, *13*, 1545−1550.
(10) (a) Weber, A.; Casini, A.; Heine, A.; Kuhn, D.; Supuran, C. T.; Scozzafava, A.; Klebe, G. Unexpected nanomolar inhibition of carbonic anhydrase by COX-2 selective Celecoxib: New pharmacological opportunities due to related binding site recognition. *J. Med. Chem.* **2004**, *47*, 550−557. (b) Pastorekova, S.; Casini, A.; Scozzafava, A.; Vullo, D.; Pastorek, J.; Supuran, C. T. Carbonic anhydrase inhibitors: The first selective, membrane-impermeant inhibitors targeting the tumor-associated isozyme IX. *Bioorg. Med. Chem.* Lett. **2004**, *14*, 869−873.
(11) (a) Vullo, D.; Voipio, J.; Innocenti, A.; Rivera, C.; Ranki, H.; Scozzafava, A.; Kaila, K.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of the human cytosolic isozyme VII with aromatic and heterocyclic sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 971−6. (b) De Simone, G.; Di Fiore, A.; Menchise, V.; Pedone, C.; Antel, J.; Casini, A.; Scozzafava, A.; Wurl, M.; Supuran, C. T. Carbonic anhydrase inhibitors. Zonisamide is an effective inhibitor of the cytosolic isozyme II and mitochondrial isozyme V: solution and X-ray crystallographic studies. *Biorg. Med. Chem. Lett.* **2005**, *15*, 2315−2320.

(12) Vullo, D.; Innocenti, A.; Nishimori, I.; Pastorek, J.; Scozzafava, A.; Pastorekova, S.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of the transmembrane isozyme XII with sulfonamides—a new target for the design of antitumor and antiglaucoma drugs? *Bioorg. Med. Chem. Lett.* **2005**, *15*, 963−9.

(13) (a) Svastova, E.; Hulikova, A.; Rafajova, M.; Zat'ovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. *FEBS Lett.* **2004**, *577*, 439−45. (b) Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastorekova, S.; Scozzafava, A.; Winum, J.-Y.; Montero, J.-L.; Supuran, C. T. Carbonic anhydrase inhibitors. Sulfonamides inhibit isozyme IX mediated acidification of hypoxic tumors. Fluorescent sulfonamides design as probes of membrane-bound carbonic anhydrase isozymes involvement in tumorigenesis. *J. Med. Chem.* **2005**, *48*, 4834−4841. (c) Menchise, V.; De Simone, G.; Alterio, V.; Di Fiore, A.; Pedone, C.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: Stacking with Phe131 determines active site binding region of inhibitors as exemplified by the X-ray crystal structure of a membrane-impermeant antitumor sulfonamide complexed with isozyme II. *J. Med. Chem.* **2005**, *48*, 5721−5727.

(14) Krungkrai**,** J.; Scozzafava, A.; Reungprapavut, S.; Krungkrai, S. R.; Rattanajak, R.; Kamchonwongpaisan, S.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of *Plasmodium falciparum* carbonic anhydrase with aromatic sulfonamides: towards antimalarials with a novel mechanism of action? *Bioorg. Med. Chem.* **2005**, *13*, 483−489.

(15) (a) Suarez Covarrubias, A.; Larsson, A. M.; Hogbom, M.; Lindberg, J.; Bergfors, T.; Bjorkelid, C.; Mowbray, S. L.; Unge, T.; Jones, T. A. Structure and function of carbonic anhydrases from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **2005**, *280*, 18782−9. (b) Suarez Covarrubias, A.; Bergfors, T.; Jones, T. A.; Hogbom, M. Structural mechanics of the pH dependent activity of beta-carbonic anhydrase from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **2006**, in press.

(16) (a) Klengel, T.; Liang, W. J.; Chaloupka, J.; Ruoff, C.; Schroppel, K.; Naglik, J. R.; Eckert, S. E.; Mogensen, E. G.; Haynes, K.; Tuite, M. F.; Levin, L. R.; Buck, J.; Muhlschlegel, F. A. Fungal Adenylyl Cyclase Integrates CO_2 Sensing with cAMP Signaling and Virulence. *Curr. Biol*. **2005**, *15*, 2021−2026. (b) Mogensen, E. G.; Janbon, G.; Chaloupka, J.; Steegborn, C.; Fu, M. S.; Moyrand, F.; Klengel, T.; Pearson, D. S.; Geeves, M. A.; Buck, J.; Levin, L. R.; Muhlschlegel F. A. *Cryptococcus neoformans* Senses CO_2 through the Carbonic Anhydrase Can2 and the Adenylyl Cyclase Cac1. *Eukaryot. Cell* **2006**, *5*, 103−111.

(17) Bahn, Y. S.; Cox, G. M.; Perfect, J. R.; Heitman, J. Carbonic Anhydrase and CO_2 Sensing during *Cryptococcus neoformans* Growth, Differentiation, and Virulence. *Curr. Biol*. **2005**, *15*, 2013−2020.

(18) (a) Marshall, B. J.; Warren, J. R. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* **1984**, *16*, 1311−1315. (b) Marshall, B. J. *Helicobacter pylori. Am. J. Gastroenterol*. **1994**, *89*, S116−S128.

(19) (a) Personnet, J.; Friedman, G. D.; Vandersteen, D. P.; Chang, Y.; Vogelman, J. H.; Orentreich, N.; Sibley, R. K. *H. pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med*. **1991**, *325*, 1131. (b) Moss, S. F.; Blaser, M. J. Mechanisms of disease: Inflammation and the origins of cancer. *Nat. Clin. Pract. Oncol.* **2005**, 2, 90−97. (c) Kountouras, J.; Zavos, C.; Chatzopoulos, D. New concepts of molecular biology on gastric carcinogenesis. *Hepatogastroenterology* **2005**, *52*, 1305−12. (d) Marshall, B. J.; Windsor, H. M. The relation of *Helicobacter pylori* to gastric adenocarcinoma and lymphoma: pathophysiology, epidemiology, screening, clinical presentation, treatment, and prevention. *Med. Clin. North Am.* **2005**, *89*, 313−44.

(20) (a) O'Mahony, R.; Vaira, D.; Holton, J.; Basset, C. *Helicobacter pylori*: current status and future prospects. *Sci. Prog*. **2004**, *87*, 269−96. (b) Megraud, F.; Marshall, B. J. How to treat *Helicobacter pylori*. First-line, second-line, and future therapies. *Gastroenterol. Clin. North Am.* **2000**, *29*, 759−73.

(21) Gisbert, J. P.; Pajares, J. M. *Helicobacter pylori* "rescue" therapy after failure of two eradication treatments. *Helicobacter* **2005**, *10*, 363−72.

(22) Nakayama, Y.; Graham, D. Y. *Helicobacter pylori* infection: diagnosis and treatment. *Expert Rev. Anti-Infect. Ther.* **2004**, *2*, 599−610.

(23) (a) Megraud, F. Basis for the management of drug-resistant *Helicobacter pylori* infection. *Drugs* **2004**, *64*, 1893−1904. (b) Loughlin, M. F. Novel therapeutic targets in *Helicobacter pylori*. *Expert Opin. Ther. Targets* **2003**, *7*, 725−735.

(24) Sachs, G.; Weeks, D. L.; Wen, Y.; Marcus, E. A.; Scott, D. R.; Melchers, K. Acid acclimation by *Helicobacter pylori*. *Physiology (Bethesda)* **2005**, *20*, 429−438.

(25) 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*, 729−738.

(26) Stahler, F. N.; Ganter, L.; Lederer, K.; Kist, M.; Bereswill, S. Mutational analysis of the *Helicobacter pylori* carbonic anhydrases. *FEMS Immunol. Med. Microbiol.* **2005**, 44, 183−189.

(27) (a) Chirica, L. C.; Elleby, B.; Lindskog, S. Cloning, expression and some properties of alpha-carbonic anhydrase from *Helicobacter pylori*. *Biochim. Biophys. Acta* **2001**, *1544*, 55−63. (b) Chirica, L. C.; Petersson, C.; Hurtig, M.; Jonsson, B. H.; Boren, T.; Lindskog, S. Expression and localization of alpha- and beta-carbonic anhydrase in *Helicobacter pylori*. *Biochim. Biophys. Acta* **2002**, *1601*, 192−199.

(28) Supuran, C. T.; Scozzafava, A.; Casini, A. Development of sulfonamide carbonic anhydrase inhibitors. In *Carbonic anhydrase—Its inhibitors and activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC Press: Boca Raton, FL, 2004; pp 67−147.

(29) ENTREZ Genome project: *Helicobacter pylori* J99 project at ASTRA; http://www.ncbi.nlm.nih.gov/entrez/query.

(30) The Institute for Genomic Research (TIGR), the Comprehensive Microbial Resource (CMR) Home Page; http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi.

(31) Tomb, J. F.; White, O.; Kerlavage, A. R.; Clayton, R. A.; Sutton, G. G.; Fleischmann, R. D.; Ketchum, K. A.; Klenk, H. P.; Gill, S.; Dougherty, B. A.; Nelson, K.; Quackenbush, J.; Zhou, L.; Kirkness, E. F.; Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H. G.; Glodek, A.; McKenney, K.; Fitzegerald, L. M.; Lee, N.; Adams, M. D.; Hickey, E. K.; Berg, D. E.; Gocayne, J. D.; Utterback, T. R.; Peterson, J. D.; Kelley, J. M.; Cotton, M. D.; Weidman, J. M.; Fujii, C.; Bowman, C.; Watthey, L.; Wallin, E.; Hayes, W. S.; Borodovsky, M.; Karp, P. D.; Smith, H. O.; Fraser, C. M.; Venter, J. C. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **1997**, *388*, 539−547.

(32) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schafer, S.; Supuran, C. T. Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfamates/sulfamides with isozymes I, II and IV. *Bioorg. Med. Chem. Lett.* **2003**, *13* (5), 841−5.

(33) Abbate, F.; Coetzee, A.; Casini, A.; Ciattini, S.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with the antipsychotic drug sulpiride. *Bioorg. Med. Chem. Lett.* **2004,** *14* (2), 337−41.

(34) Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: E7070, a sulfonamide anticancer agent, potently inhibits cytosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217−23.

(35) Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. Carbonic anhydrase inhibitors. Inhibition of tumor-associated isozyme IX by halogenosulfanilamide and halogenophenylaminobenzolamide derivatives. *J. Med. Chem.* **2003**, *46*, 2187−96.

(36) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. Carbonic anhydrase inhibitors: synthesis of water-soluble, aminoacyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J. Med. Chem.* **1999**, *42*, 3690−700.

(37) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641−50.

(38) Supuran, C. T.; Clare, B. W. Quantum theoretic QSAR of benzene derivatives: some enzyme inhibitors. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 237−48.

(39) Winum, J. Y.; Dogne, J. M.; Casini, A.; de Leval, X.; Montero, J. L.; Scozzafava, A.; Vullo, D.; Innocenti, A.; Supuran, C. T. Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/membrane-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating hydrazino moieties. *J. Med. Chem.* **2005**, *48*, 2121−5.

(40) Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. Carbon-13 nuclear magnetic resonance probe of active-site ionizations in human carbonic anhydrase B. *Biochemistry* **1977**, *16*, 2241−7.

(41) 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*, 2561−73.

(42) (a) Puscas, I. Carbonic anhydrase and inhibition of gastric secretion by acetazolamide. *Med. Interna* **1973**, *25*, 1243−1252. (b) Puscas, I.; Paun, R.; Ursea, N.; Dragomir, N.; Roscin, M. Les inhibiteurs de

l'anhydrase carbonique dans le traitement de l'ulcere gastrique et duodenal. *Arch. Fr. Mal. Appar. Dig.* **1976**, *65*, 577−583. (c) Puscas, I. Treatment of gastroduodenal ulcers with carbonic anhydrase inhibitors. *Ann. N.Y. Acad. Sci.* **1984**, *429*, 587−591. (d) Puscas, I. Treatment of gastroduodenal ulcers with inhibitors of gastric mucosa carbonic anhydrase. In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*; Puscas, I., Ed.; Helicon Press: Timisoara, Romania, 1994; pp 438−450.

(43) Maren. T. H. Carbonic anhydrase: Chemistry, physiology and inhibition. *Physiol. Rev.* **1967**, *47*, 595−781.

(44) Sano, S.; Nishimori, I.; Kosaki, T.; Fujikawa-Adachi, K.; Onishi, S. The fine form of *Helicobacter pylori* on the metaplastic duodenal mucosa is associated with recurrent duodenal ulcers. *Digestion* **2001**, *64*, 161−8.

JM0512600