Structure-Activity Relationship of Omeprazole and Analogues as *Helicobacter pylori* Urease Inhibitors

Thomas C. Kühler,*,† Jan Fryklund,‡ Nils-Åke Bergman,† Jessica Weilitz,‡ Adrian Lee,§ and Håkan Larsson⊥

Departments of Medicinal Chemistry, Cell Biology, and GI Pharmacology, Astra Hässle AB, 431 83 Mölndal, Sweden, and School of Microbiology, University of New South Wales, P.O. Box 1, Kensington, New South Wales, Australia

Received April 24, 1995®

Helicobacter pylori urease belongs to a family of highly conserved urea-hydrolyzing enzymes. A common feature of these enzymes is the presence of two Lewis acid nickel ions and a reactive cysteine residue in the active site. The H^+/K^+-ATPase inhibitor omeprazole is a prodrug of a sulfenamide which covalently modifies cysteine residues on the luminal side of the H^+/K^+-ATPase of gastric parietal cells. Omeprazole and eight analogues were selected based on their chemical, electronic, and kinetic properties, and each was incubated with viable *H. pylori* in phosphate-buffered saline at pH 7.4 for 30 min, after which 100 mM urea was added and the amount of ammonia formed analyzed after a further 10 min. Inhibition between 0% and 100% at a 0.1 mM concentration was observed for the different analogues and could be expressed as a function of the pK_a-value of the pyridine, the pK_a-value of the benzimidazole, the overall lipophilicity, and, most importantly, the rate of sulfenamide formation, in a quantitative structure-activity relationship. The inhibition was potentiated by a lower pH (favoring the formation of the sulfenamide) but abolished in the presence of β-mercaptoethanol (a scavenger of the sulfenamide). Structural analogues incapable of yielding the sulfenamide did not inhibit ammonia production. Treatment of *Helicobacter felis*-infected mice with 230 μmol/kg flurofamide b.i.d. for 4 weeks, known to potently inhibit urease activity *in vivo*, as a means of eradicating the infection, was tested and compared with the effect of 125 μmol/kg omeprazole b.i.d. for 4 weeks. Neither treatment proved efficacious.

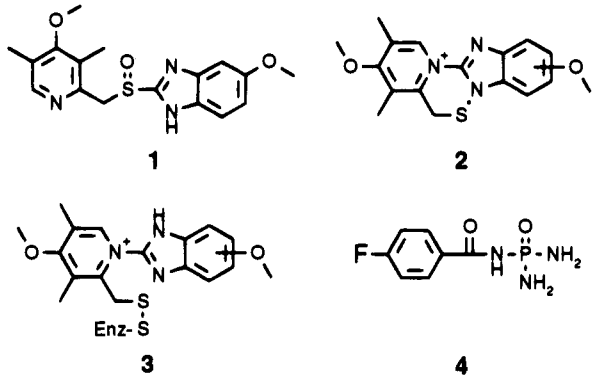
Introduction

Helicobacter pylori plays an important role in peptic ulcer disease,[1,2] and eradication of the bacterium in patients with peptic ulcers dramatically decreases the risk of ulcer recurrence.[3-5] The obvious remedy for treating a bacterial infection with antimicrobials, however, has often proven futile,[6] and only a few combination regimens have reached clinical practice.[7,8] Thus, the need for alternative or novel treatments is evident. The possibility of development of resistance is another strong argument for identifying new drugs directed against *H. pylori*.

H. pylori provides a unique nonmammalian target in its urease, EC 3.5.1.5.[9] The bacterium allocates a considerable proportion of its anabolic activity toward the production of urease since it seemingly is constitutively expressed[10] and amounts to as much as 6% of the soluble protein.[11] The enzyme is required during the colonization of gnotobiotic piglets[12] and SPF mice.[13] Indeed, marmosets infected in the oropharnyx with ureaplasmas are successfully cured by interfering with the urease of the microbes, whereas treatment with conventional antibiotics merely leads to a stasis of the infection.[14]

In the pioneering work on the archetype urease from *Canavalia ensiformis* (Jack bean), Zerner demonstrated the presence of two Lewis acid nickel ions and more importantly a reactive cysteine residue in the active site of the enzyme.[15] Others have later reported on *H. pylori* urease having significant amino acid sequence similarity with other microbial ureases as well as with Jack bean urease,[11] suggesting a common ancestral urease gene and thus similar active sites.

Chart 1. Structures of Omeprazole (**1**), the Active Sulfenamide **2**, the Enzyme-Inhibitor Disulfide Complex **3**, and Flurofamide (**4**)



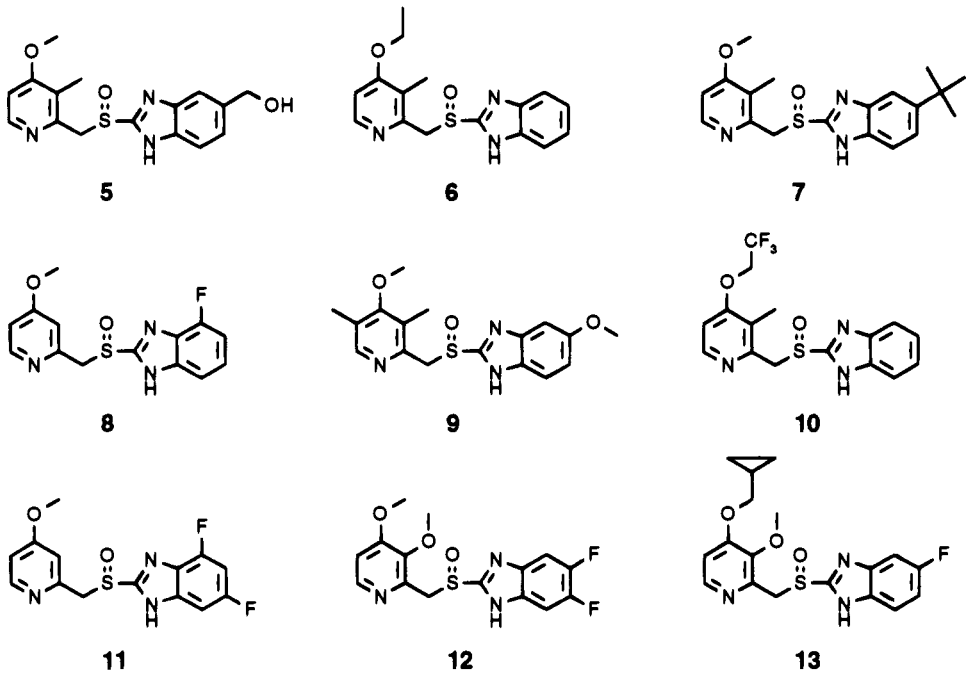
The potent antiulcer agent omeprazole (**1**) is a prodrug of the sulfenamide **2** and exerts its effect by covalently modifying cysteine residues **3** on the luminal side of the proton pump,[16] *i.e.*, the H^+/K^+-ATPase of the parietal cell in the oxyntic mucosa of the stomach.

Omeprazole (**1**) is converted to the sulfenamide **2** only at low pH. In the (human) body this is exclusively achieved in the tubulovesicular and canalicular structures of the parietal cell, *i.e.*, the structures carrying the proton pump. Compound **1** is also a weak base rendering it to accumulate in these acidic compartments. In addition, once there, it is rapidly transformed to the sulfenamide **2**, a charged species, which ef-

* Author for correspondence at Medicinal Chemistry, Astra Hässle AB, 431 83 Mölndal, Sweden. Tel: +46-31-766 1362. Fax: +46-31-776 3724.
† Department of Medicinal Chemistry, Astra Hässle AB.
‡ Department of Cell Biology, Astra Hässle AB.
⊥ Department of GI Pharmacology, Astra Hässle AB.
§ University of New South Wales.
® Abstract published in *Advance ACS Abstracts,* October 15, 1995.

0022-2623/95/1838-4906$09.00/0

Chart 2. Omeprazole (**9**) and the Structural Analogues Selected To Be Checked for Urease Inhibition[a]



[a] The stability of the compounds (prodrugs) increases rowwise from the top to the bottom, whereas the lipophilicity increases columnwise from the left to the right. Compound **10** is lansoprazole.

Table 1. Physicochemical Data and the Identity Number Used in the PLS Model of the Compounds Studied[a]

compd	PLS id no.	$pK_{a_{py}}$	$pK_{a_{bz}}$	log k'_o	$t_{1/2}$ (min)
5	1	4.7	8.6	0.18	60
6	2	4.9	8.8	0.92	54
7	3	4.8	9.1	1.55	60
8	4	4.4	7.5	0.26	1380
9	5	4.0	8.7	0.83	1380
10	6	3.9	8.7	1.10	780
11	7	4.1	7.6	0.09	1920
12	8	3.6	7.8	0.60	4140
13	9	3.8	8.4	1.16	3000
9 at pH 6	10	4.0	8.7	0.83	55
37	11	5.1	8.9	1.22	90
38	12	4.9	9.4	1.21	70

[a] The lipophilicity is expressed as chromatographic retention, k'_o. The half-life, $t_{1/2}$, is a measure of the rate by which each compound (prodrug) is converted to the active species.

fectively prevents it from reentering the systemic circulation, trapping it close to the target enzyme. This gives a selective and specific mode of action in the human body.[17]

The objectives of this study were to (i) demonstrate that omeprazole and structural analogues (after initial conversion to active sulfenamides) could inhibit *H. pylori* urease *in vitro* by covalently modifying cysteine residues in the enzyme, (ii) establish a structure–activity relationship (SAR) if possible, (iii) optimize potency according to the SAR, and (iv) investigate if urease inhibition *in vivo* could be a means of killing the bacterium.

Chemistry

Design Concepts. Nine omeprazole analogues, *cf.* Chart 2, were selected based on the half-life at pH 7 and the overall lipophilicity expressed as chromatographic retention, *cf.* Table 1. Compounds with a fast ($t_{1/2,pH7}$ < 100 min), an intermediate (13 h < $t_{1/2,pH7}$ < 25 h), and a slow ($t_{1/2,pH7}$ > 35 h) conversion rate were chosen. Within each group a hydrophilic, an intermediate, and a lipophilic compound were selected.

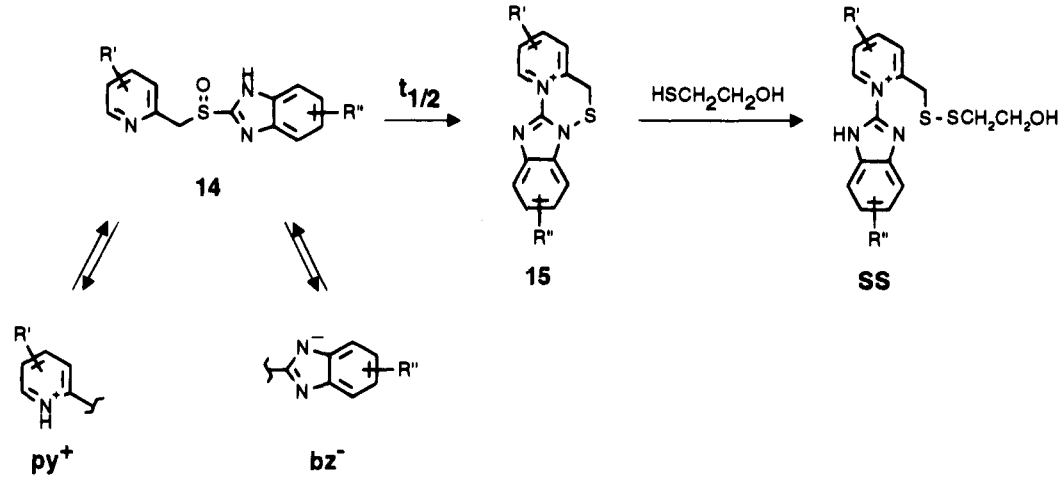
The compounds are amphoteric, and charged species will to different extents contribute to the lipophilicity, *cf.* Scheme 1. A means of estimating the contribution of these charged species to the overall lipophilicity is chromatographic retention time, k'.[18] Relating each retention time to a reference compound (**9**) has the advantage of compensating for variations between different experiments as well as yielding relative[19] (k'_o) rather than absolute lipophilicities.

The half-life reflects the rate of conversion from prodrug **14** to the active species, the sulfenamide **15**, and is very dependent on the electronic properties of the substituents on both the pyridine and the benzimidazole rings.[20] The electronic properties of the substituents R′ and R″ are accounted for in their contribution to the pK_a-values and thus to the half-life ($t_{1/2}$), and their lipophilicity is accounted for in their contribution to the relative retention time (k'_o). Their steric properties were, in this context, judged to be of minor importance and thus disregarded.

The four variables (the logarithm of) k'_o, pK_{apy}, pK_{abz}, and $t_{1/2}$ were used to establish a quantitative structure–activity relationship (QSAR) model by the partial least squares (PLS) method.[21] This model, based on nine entries, was then used to make predictions of the activity of three additional compounds, two of which were synthesized and tested.

Syntheses. The sulfoxides **16** were obtained after oxidizing the corresponding sulfides **17** with *m*-CPBA,[22] *cf.* Scheme 2. The sulfides **17** were prepared by alkylating the appropriate mercaptobenzimidazole **18** with the appropriate (chloromethyl)pyridine **19**. Substituted 2-methylpyridines **20** were oxidized with *m*-CPBA to furnish the *N*-oxides **21** which could readily be activated in the 4-position by nitration (**22a**). The alkyloxy compounds **23** were usually obtained after treatment

Scheme 1. Prodrug **14** Converts to the Active Species, the Sulfenamide **15**, at a Rate Dependent on the Half-Life, $t_{1/2}$[a]



[a] An excellent scavenger of the sulfenamide is β-mercaptoethanol furnishing a disulfide adduct (SS). The pK_a-values of the prodrug are dependent on the electronic properties of the substituents R' and R", and charged species (py$^+$ and bz$^-$) will to different extents contribute to the overall lipophilicity (of the parent compound).

of **11a** with alkoxide ion, but on occasion the chloro compound **22b** had to be prepared in order to modify reactivity. Reaction with acetic anhydride yielded the acetyl derivatives **24**, which could be rearranged according to Katada,[23] furnishing 2-[(acetyloxy)methyl]-pyridines (**25**). These were hydrolyzed with sodium hydroxide, leaving hydroxymethyl compounds **26** which upon treatment with thionyl chloride gave the chloro compounds **19**.

Not all the 2-methylpyridines (**20**) needed were commercially available but had to be synthesized. 3-Hydroxy-2-methyl-4-pyrone (**27**) was alkylated to furnish **28**, treated with ammonia to yield the corresponding pyridone **29**, chlorinated with $POCl_3$ to leave **30**, and treated with alkoxide ion to yield **31**. The *N*-oxides **23** were prepared and subjected to the Katada conditions as discussed above.

The mercaptobenzimidazoles **18** were obtained by reacting potassium ethyl xanthogenate with phenylenediamines **32**. The diamines were obtained by catalytic hydrogenation of the corresponding nitroanilines **33** obtained by consecutive nitrations and reductions of properly substituted benzenes, anilines, or nitrobenzenes.

Pharmacology

Bacterial Strains. The *H. pylori* strains used were CCUG 15818 and N6Km-5. N6Km-5 was a urease negative mutant constructed from N6, a strain originating from patients with gastritis. It was formed by allelic exchange in the *ureB* gene, and it does not synthesize the *ureB* product.[24] The *Helicobacter felis* strain used was CS1.

N6Km-5 was kindly provided by Dr. Agnès Labigne (Institut Pasteur, Paris, France) and CS1 by Professor Adrian Lee (University of New South Wales, Sydney, Australia). The CCUG strain was obtained from the Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Sweden.

Stock cultures were stored at −70 °C in Brucella broth with 10% fetal calf serum (Difco) supplemented with 20% glycerol at pH 7. The serum was inactivated at 56 °C for 30 min prior to use. Colombia blood agar was used as solid medium for *Helicobacter* specimens: 42.5 g/L Colombia agar base II (Oxoid), 15 g/L bactoagar (Oxoid), 7% horse blood, and 1% IsoVitaleX (BBL Microbiology System), pH 7.3 +/−0.2.

Urease Assay. The test compounds were incubated with intact *H. pylori*, intact *H. felis*, or purified Jack bean urease (Sigma) at various concentrations. Urea was added, and the amount of ammonia formed was determined after 10 min, employing a modified phenol hypochlorite reaction[25] catalyzed by sodium nitroferricyanide to form a blue indophenol.[26] Color development of the test compounds was determined in control experiments without bacteria. When such effects were noticed, the control value was subtracted from the actual experimental result. The rate of urea hydrolysis was found to be linear in the 10 min interval studied.

***In Vivo* Studies.**[27] The *in vivo* model used in this study has been described earlier,[28] but briefly SPF mice were challenged with *H. felis* three times during a 6 day period, and 3 weeks after inoculation animals were treated orally according to different regimens for 4 weeks.

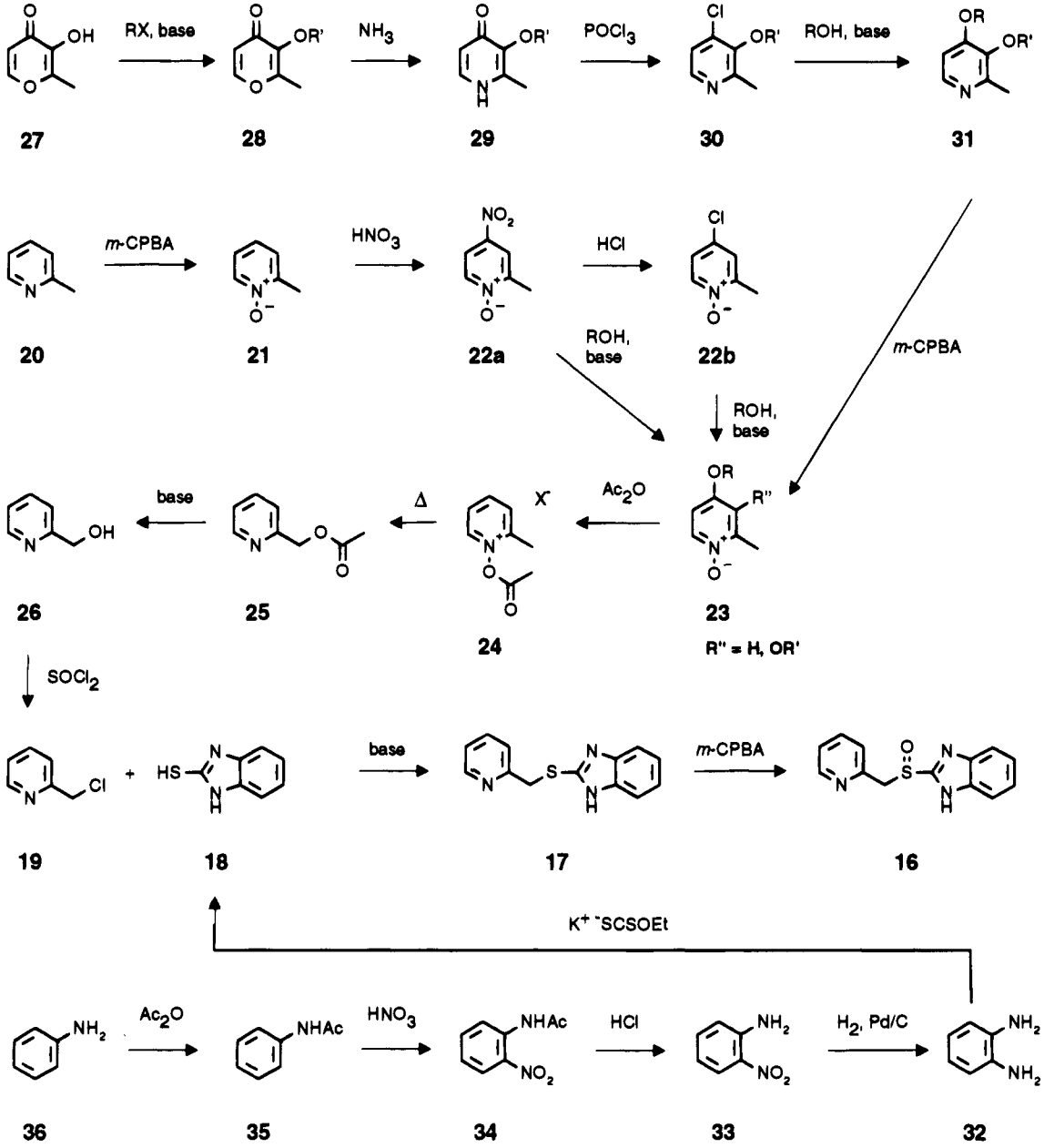
Six different regimens were selected as follows: an uninfected no treatment control, an infected no treatment group to check for spontaneous elimination of the infection, an infected group receiving vehicle only, a triple therapy group used as a positive eradication control, and, finally, the two groups to be studied, a high-dose omeprazole and a therapeutic dose flurofamide, *vide infra*.

Animals were treated for 4 weeks and sacrificed 24 h or 5 weeks after cessation of the treatment to measure suppression and eradication, respectively. The assessment was done by checking mouse stomach specimens for urease activity,[29] and the rate of both suppression and eradication for each regimen was expressed as the number of urease positive animals divided by the number of animals checked × 100%.

Results

***In Vitro* Studies.** Production of ammonia by urease in the urease positive *H. pylori* strain CCUG 15818 was

Scheme 2. Synthetic Routes Employed toward the Desired Compounds **16**[a]



[a] Substituents are not shown in structures **16**–**19**, **24**–**26**, and **32**–**34** for reasons of clarity. The reader is referred to Charts 2 and 3 to check for actual substituents and compounds prepared.

compared with ammonia production in the urease negative *H. pylori* strain N6Km-5. After 10 min no detectable ammonia had been formed by the urease deficient strain (A_{560} = 0.24), whereas 1.44 mM ammonia had been formed by the urease positive strain (result not shown). Flurofamide (**4**), *cf.* Chart 1,[30] the most potent urease inhibitor yet described, was used to validate the assay, and it inhibited ammonia production completely at 100 nM and partly even at 10 and 1 nM concentrations, Figure 1.

The effects of omeprazole and analogues at 1, 10, and 100 μM concentrations along with that of flurofamide at 1, 10, and 100 nM concentrations on *H. pylori*-associated urease activity are given in Table 2 and expressed as percent of control. Table 2 also shows that the inhibitions at 1 and 10 μM are very low (production of ammonia very close to that of the controls) for most of the compounds. These data are thus not ideal as *y*-variables in a PLS analysis. Using the activities of

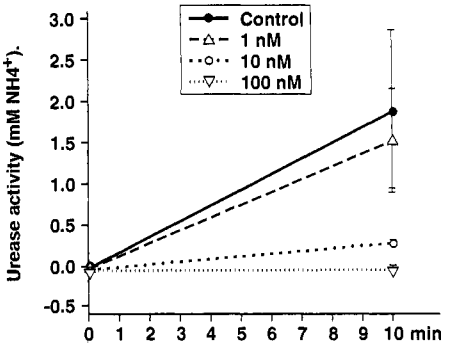


Figure 1. Flurofamide (**4**) dose dependently inhibits ammonia production in wild-type *H. pylori* strain CCUG 15818.

the compounds corresponding to entries 1–7, 9, and 10 at 100 μM together with the four variables $pK_{a_{py}}$, $pK_{a_{bz}}$, log k'_0, and $t_{1/2}$, however, a PLS model could be established, Figure 2. Only the first PLS component was significant according to cross-validation, but the predic-

Table 2. Urease Activities in Wild-Type *H. pylori*, CCUG 15818, When Subjected to Different Concentrations of Test Compounds[a]

compd	PLS id no.	concentration (μM) 1	10	100
5	1	72	38	32
6	2	76	38	8
7	3	105	42	1
8	4	93	95	58
9	5	109	96	64
10	6	105	103	29
11	7	106	89	80
12	8	ND	ND	ND
13	9	104	94	84
9 at pH 6	10	115	88	28
37 (calcd)				0
37 (obsd)	11	86	35	0
38 (calcd)				0
38 (obsd)	12	36	35	0
7 + β	13	105	95	106
39	14	109	101	99
40	15	97	105	164
flurofamide (**4**)		80 (1 nM)	10 (10 nM)	0 (100 nM)

[a] The results are expressed as percent of control. The PLS model predicted two compounds, **37** and **38**, to be potent inhibitors. The calculated values (calcd) are given along with the observed ones (obsd). The symbol β denotes β-mercaptoethanol. ND, not determined.

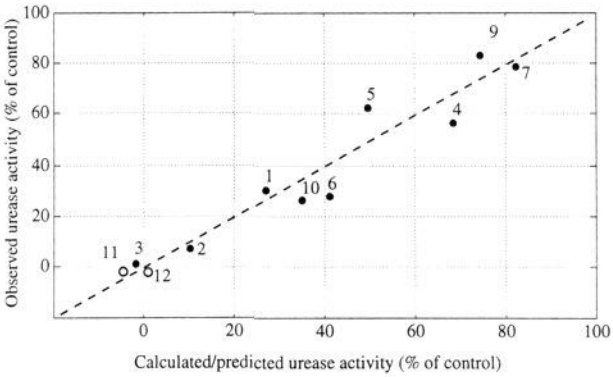
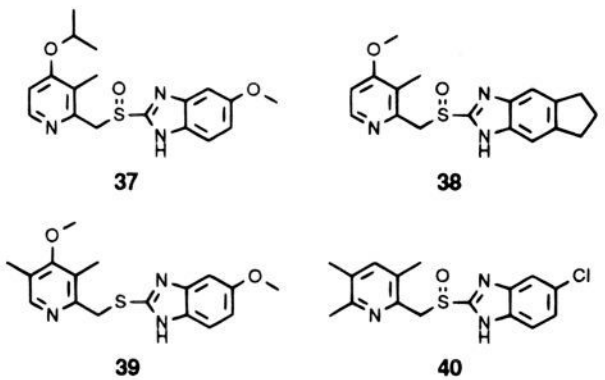


Figure 2. PLS model showing observed versus calculated/predicted urease activities expressed as percent of control for some omeprazole analogues. The PLS model is based on entries 1–7, 9, and 10 (●). Entries 11 and 12 are predicted (○) and confirmed experimentally.

Chart 3. Structures of the Two Compounds Predicted by the PLS Model To Be Potent Urease Inhibitors (Top row) and Structural Analogues Incapable of Yielding a Sulfenamide Structure (Bottom row)



tive power of this simple model was good (Q^2 = 0.85). Examination of the PLS regression coefficients showed that, in order to maximize urease inhibition, $t_{1/2}$ should attain the lowest possible numerical value, whereas

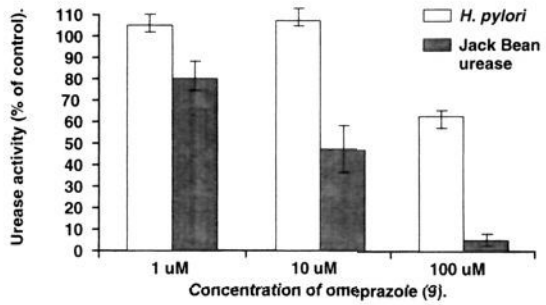


Figure 3. Comparison of the effects of omeprazole (**9**) on the urease activity in wild-type *H. pylori* strain CCUG 15818 (n = 4) and purified Jack bean urease (n = 3) at pH 7.5.

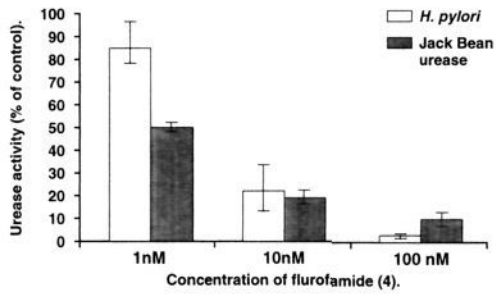


Figure 4. Comparison of the effects of flurofamide (**4**) on the urease activity in wild-type *H. pylori* strain CCUG 15818 (n = 2) and purified Jack bean urease (n = 3) at pH 7.5.

$pK_{a_{py}}$, $pK_{a_{bz}}$, and log k'_o should attain as big numerical values as possible. The single most important variable proved to be $t_{1/2}$ and the least important one log k'_o.

The PLS model was used to predict, and improve, the activities of new compounds, *cf.* Chart 3. Thus compounds **37** and **38** (entries 11 and 12, respectively), with $t_{1/2}$ < 100 min, $pK_{a_{py}} \approx 5$, $pK_{a_{bz}} \approx 9$, and log k'_o > 1, were synthesized and tested. For both compounds the predicted values accorded very well with the experimentally determined ones, *cf.* Table 2 and Figure 2. Compound **12** (entry 8) was predicted to have poor activity. For reasons given in the Discussion section, this compound was never tested.

The inhibition was potentiated by a lower pH (entry 10), favoring the formation of the sulfenamide **15**,[20] but entirely abolished in the presence of 100 μM β-mercaptoethanol (entry 13), a known scavenger of the sulfenamide,[20] *cf.* Scheme 1. Structural analogues incapable of yielding the sulfenamide **15**, *e.g.*, the sulfide **39** (entry 14) and the 6-methyl derivative **40**[31] (entry 15), did not inhibit ammonia production, *cf.* Chart 3 for structures.

To verify that omeprazole and analogues inhibit *H. pylori* ammonia production by an interaction with the urease rather than by nonspecific cell-wall interaction, the inhibition was (i) checked on purified Jack bean urease and (ii) compared with that of flurofamide (an active site blocker), Figures 3 and 4. Omeprazole significantly inhibited purified Jack bean urease more than *H. pylori*-associated urease, Figure 3. Flurofamide too, was a more potent inhibitor of Jack bean urease than *H. pylori*-associated urease at a 1 nM concentration but a less potent inhibitor at a 100 nM concentration, Figure 4.

Regarding the optimized compound **38**, it inhibited purified Jack bean urease to about the same extent as omeprazole but was indeed a more potent inhibitor of *H. pylori*-associated urease, *cf.* Figures 3 and 5.

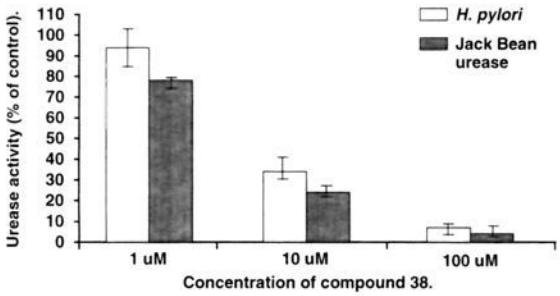


Figure 5. Comparison of the effects of compound **38** on the urease activity in wild-type *H. pylori* strain CCUG 15818 ($n = 3$) and purified jack bean urease ($n = 3$) at pH 7.5.

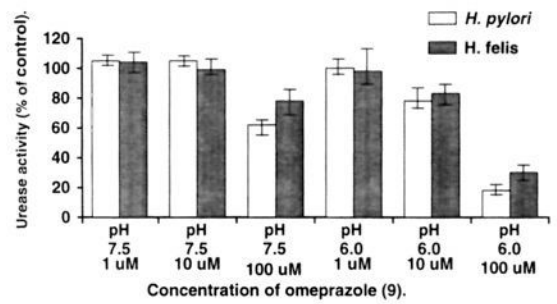


Figure 6. Comparison of the effects of omeprazole (**9**) on the urease activity in wild-type *H. pylori* strain CCUG 15818 and wild-type *H. felis* strain CS1 at pH 6.0 and 7.5; $n = 3$ with the exception of entry *H. pylori*, pH 7.5, where $n = 4$.

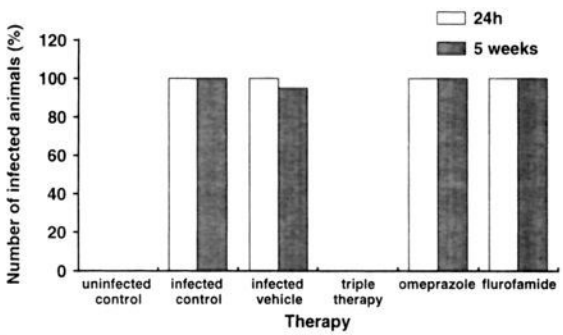


Figure 7. *H. felis* (CS1) status in various control and treated SPF mice 24 h and 5 weeks after stopping treatment. The suppression and eradication rates in each group are expressed as the number of urease positive animals divided by the number of animals checked × 100% (at least 10 in each instance).

Although our aim was never to determine any IC_{50}-values for omeprazole, inspection of Figure 3 and Table 2 (entry 5) suggests IC_{50}-values of close to 10 μM and slightly in excess of 100 μM for inhibition of purified (Jack bean) urease and *H. pylori*-associated urease, respectively.

***In Vivo* Studies.** For the *in vivo* studies, the effect of omeprazole (**9**) on *H. pylori* ammonia production was compared with the effect on *H. felis* ammonia production at both pH 7.5 and pH 6.0 and found to be about the same, Figure 6. The experiments were performed with the same optical density, at which there was more *H. felis* than *H. pylori*, however.

Not a single animal in the *H. felis*-infected control group spontaneously eliminated the infection nor did uninfected controls become infected, Figure 7. Treatment with vehicle alone did not affect the *H. felis* status of the animals, whereas triple therapy was 100% efficacious in both suppression and eradication. Neither omeprazole, flurofamide, nor the combination of the two (result not shown) caused any suppression or eradication of the infection.

Discussion

Omeprazole inhibits isolated H^+/K^+-ATPase, under neutral conditions when only very little active sulfenamide is formed, with an IC_{50} of 2 μM,[17] which is in the same order of magnitude as our tentative IC_{50}-value of about 10 μM for inhibition of purified Jack bean urease (also studied under neutral conditions). However, when omeprazole is studied in gastric gland preparations (acid conditions, $IC_{50} = 0.6$ μM)[32] and compared with the effect on urease associated with competent bacteria (neutral conditions, tentative $IC_{50} \geq 100$ μM), the need for a low pH for activation of the prodrug, and thus the selectivity of omeprazole, is nicely illustrated. Another enzyme also carrying essential cysteine residues such as the Na^+/K^+-ATPase is not at all inhibited by omeprazole unless studied under acid conditions when conversion to the active sulfenamide is promoted, again demonstrating the need for a low pH for activation of the drug.

Preincubation of omeprazole for 30 min with partly purified *H. pylori* urease has been reported to give a reduction in urease activity of 87% at 150 μM and 75% at 75 μM.[33] These values compare well with ours observed on *H. pylori*-associated urease, 64% at 100 μM. However, we do not agree with the conclusion that omeprazole competitively inhibits urease. On the contrary, our data strongly suggest a covalent modification of essential cysteine residues (SH groups) in the urease and thus a noncompetitive inhibition, *e.g.*, (i) the more sulfenamide formed (*i.e.*, shorter $t_{1/2}$ and/or a lowered pH) the better the inhibition, (ii) coincubation with β-mercaptoethanol effectively scavenges the sulfenamide and preserves urease activity, and (iii) structural analogues incapable of yielding a sulfenamide lack inhibitory effect. This suggestion is corroborated by Nagata *et al.*[34] who studied the effects of omeprazole and lanzoprazole (compound **10** in this study) on urease. They similarly demonstrated that inhibition was potentiated at a lower pH but abolished in the presence of SH-containing compounds such as glutathione or dithiothreitol. Indeed, recently Jabri *et al.* published the X-ray structure of *Klebsiella aerogenes* urease[35] and showed the presence of a cysteine residue close to the putative urea-binding pocket at a position where chemical modification, *e.g.*, disulfide formation with sulfenamides, would lead to steric blockage of the active site.

The effects of flurofamide, omeprazole, and the more potent compound **38** on ammonia production by *H. pylori* and purified Jack bean urease were compared. The inhibitory effects differed but were within 1 order of magnitude roughly the same for the different compounds on both the bacterium (*H. pylori*) and the purified protein (urease). This lends support to the notion that omeprazole and structural analogues inhibit *H. pylori* ammonia production by interfering with the bacterial urease, at the active site as discussed above, rather than by an unspecific modification of bacterial surface proteins. In accord with this, the number of available SH groups in water-extracted *H. pylori* urease decreases upon treatment with lansoprazole.[34]

Since the *in vivo* studies were to be done on *H. felis* rather than on *H. pylori*-infected animals, the effect of

omeprazole was checked in both species and established to be identical. Further, the potent urease inhibitor flurofamide had been shown to inhibit urease activity in ferrets infected with *Helicobacter mustele* by some 95–100% for 24 h after a single oral dose of 23 μmol/kg[36] and was thus considered valid as a control treatment.

In addition to urease inhibition, omeprazole does affect (bacterial) cell viability *in vitro*.[37] The bactericidal effect, however, is not urease mediated since there is no difference in the susceptibility of the wild-type *H. pylori* (N6) or the corresponding urease deficient mutant (N6Km-5) to omeprazole.[37] Yet, despite the possibility of a double pressure, *i.e.*, bactericidal activity and urease inhibition, a course of omeprazole did not clear, let alone eradicate, the *Helicobacter* infection in mice. This result does not differ from that obtained with flurofamide which potently inhibits urease both *in vitro* and *in vivo*[36] (but does not affect cell viability of either the wild-type *H. pylori* strain or the urease deficient mutant *in vitro*[37]).

Although we have not established if omeprazole indeed does inhibit urease activity in the *in vivo* situation, or excerts an antibacterial effect *in vivo* for that matter, the work on preparing even more potent urease inhibitors extrapolating from the dataset on which the PLS model was built could not be justified any more. Also, with the *in vivo* results at hand, the work on bringing up enough material of compound **12** to be tested was discontinued. Besides, the PLS model had proven to have enough predictive power even without the data for this compound.

Regarding the initial statement, that the steric bulk of the substituents R′ and R″ could be disregarded, we feel it proved valid, since the PLS model became predictive with a Q^2-value of 0.85. The significance of the findings that the most important variable was the half-life and that the lipophilicity was of only minor importance, though it should be as high as possible, can merely be hypothesized on. A lipophilic compound may penetrate cell membranes or diffuse deep into protein cavities. A sulfenamide, however, is a charged species and is not thought to penetrate cell membranes. Our division of both the half-life and the lipophilicity into only three coarse groups does not allow us to tell if there is an optimal relationship between the two variables. Nevertheless, our data are in accordance with the finding that a significant portion of the urease of bacteria cultured *in vitro* is found extracellularly and/or superficially in the periplasmic space.

With respect to our objectives we have (i) demonstrated that omeprazole and structural analogues (after initial conversion to active sulfenamides) indeed inhibit *H. pylori* urease *in vitro* by covalently modifying essential cysteine residues in the enzyme, (ii) established a QSAR, (iii) used the QSAR to predict and, to some extent also, optimize potency according to it, and (iv) checked our biological hypothesis that urease inhibition could be a means of killing the bacterium *in vivo* and proven that this is not the case.

Experimental Section

Chemicals, reagents, solvents, and drugs were purchased from any of the major vendors if nothing else is stated or referenced.

NMR spectra were recorded on 300 or 500 MHz instruments manufactured by Bruker, Switzerland. Absorbance was determined with a T_{max} microtiter plate reader from Molecular Devices Co., Menlo Park, CA.

Chemistry. The following standard preparative procedures were used.

Method I: Compounds 19 from Compounds 23. This reaction sequence was performed on a 5–30 mmol scale. The appropriate pyridine *N*-oxide **23** (x mmol) was dissolved in $2x$ mL of acetic anhydride and heated to 100 °C for 4 h. The excess acetic anhydride was evaporated, after which the residue was dissolved in absolute EtOH and evaporated to remove remaining traces of acetic acid. The latter procedure was repeated twice leaving a nearly quantitative amount of **25** as a brownish oil reasonably pure as judged by TLC.

The oil was dissolved in $1.5x$ mL of 2 M NaOH and heated to 100 °C for 1 h. After cooling, the aqueous reaction mixture was extracted twice with 50 mL of CH_2Cl_2, dried over $MgSO_4$, and evaporated leaving some 90% of the theoretical amount of **26** as a yellow oil which on occasion spontaneously crystallized.

Compound **26** (y mmol) was dissolved in $3y$ mL of CH_2Cl_2 and treated with $1.3y$ mmol of $SOCl_2$ for 2 h. The solvent and the reagent were evaporated leaving a quantitative yield of **19** as the hydrochloride.

Method II: Compounds 18 from Compounds 36. The appropriately substituted aniline **36** was acetylated with acetic anhydride at 110 °C for 2 h (about 0.5 mol/200 mL of acetic anhydride). After cooling, the reaction mixture was poured onto ice–water and the precipitated product collected. The filter cake was washed twice with water and dried *in vacuo* to leave some 70–80% of the theoretical amount of **35**.

The acetanilide **35** was added to a 4× molar excess of fuming nitric acid cooled to 0 °C, at such a rate that the temperature could be kept below 5 °C.[38] After completion of the addition, the reaction mixture was poured onto ice–water and the precipitated product collected. The moist solid material could usually be crystallized from EtOH furnishing **34** in varying yields.

Compound **34** (z mmol) was hydrolyzed with z mL of 9 M HCl at reflux for 30 min. After cooling, the slurry was treated with saturated NaOH until pH 11. The precipitate was collected, washed with water, and dried *in vacuo* to furnish an almost quantitative yield of **33**.

A suspension of **33** (k mmol) in $2k$ mL of EtOH was hydrogenated over Pd/C at ambient temperature. After completion of the reaction, a few spatula spoons of Celite was added and the solid material filtered off.[39] The filtrate was diluted with k mL of EtOH, and a solution of $K^+{}^-SCSOEt$ ($1.25k$ mmol) in $0.5k$ mL of H_2O was added and allowed to react at reflux for 12 h. The reaction mixture was diluted with water and the pH adjusted to *ca.* 7. Evaporation of most of the EtOH caused the product to precipitate, which was collected, washed with water, and dried *in vacuo* leaving varying yields of **18** pure enough to be used without further purification.

Method III: Compounds 16 from Compounds 18 and 19. These reactions were usually carried out on a maximum 5 mmol scale. Thus, **18** ($1.1m$ mmol) was suspended in $7m$ mL of MeOH and treated with NaOH ($2.0m$ mmol dissolved in a minimum volume of water) to furnish a solution. The mixture was treated with **19** ($1.0m$ mmol) and allowed to react until completion as judged by TLC (95/5 CH_2Cl_2/MeOH) at ambient temperature or reflux.[40] The solvents were evaporated, and the residue was taken up in 30 mL of 2.5% NaOH. The aqueous layer was extracted with 50 + 25 mL CH_2Cl_2; the organic layers were combined, dried over $MgSO_4$, and evaporated, leaving a good yield of **17** as a tanned foam.[41]

A stirred solution of **17** (n mmol) in $10n$ mL of CH_2Cl_2 layered with $5n$ mL of H_2O containing $NaHCO_3$ ($2n$ mmol) was cooled to 0 °C and treated with *m*-CPBA (n mmol) dissolved in a few milliliters of CH_2Cl_2. The reaction was allowed to proceed for 10 min, after which the organic layer was collected and extracted with alkaline water ($2n$ mmol of NaOH/$10n$ mL of H_2O). The alkaline extract was depressurized for a few minutes on a Rotavapor (to remove remaining traces of

CH_2Cl_2) and then treated with MeOOCH (2*n* mmol) for some 20 min. At most instances the desired product **16** would start to precipitate as a white solid.[42] The solid was collected, washed with water, and dried *in vacuo* leaving varying amounts of pure **16**.

5-(Hydroxymethyl)-2-[[(4-methoxy-3-methyl-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (5). Step 1. The synthesis of the title compound required access to 4-methoxy-2,3-dimethylpyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-4-methoxy-3-methylpyridine.

Thus, 2,3-dimethylpyridine (45 g, 0.45 mmol) was dissolved in 150 mL of concentrated acetic acid, mixed with 25 mL of 30% H_2O_2, and gently heated to 95 °C for 3 h. A second lot of 11 mL of 30% H_2O_2 was added and allowed to react for an additional 12 h. The solvent was evaporated[43] and the residue recrystallized from isopropyl ether leaving 44 g (80%) of 2,3-dimethylpyridine *N*-oxide.

The *N*-oxide (44 g, 0.36 mmol) was dissolved in 90 mL of concentrated H_2SO_4 and treated with a mixture of 130 mL of concentrated H_2SO_4 and 150 mL of 65% HNO_3. The mixture was heated to 95 °C for 20 h. The mixture was cooled and treated with 10 M NaOH until pH 3. The precipitate was collected and crystallized from EtOH yielding 32 g (53%) of 2,3-dimethyl-4-nitropyridine *N*-oxide.[44]

The nitro compound (36 g, 0.21 mol) was dissolved in 450 mL of MeOH and treated with 33 g of K_2CO_3 at reflux for 2 h. After cooling and filtration, the solvent was evaporated. The residue was taken up in CH_3CN, filtered, and reevaporated leaving 32 g (99%) of 4-methoxy-2,3-dimethylpyridine *N*-oxide.

Step 2. The synthesis of the title compound also required access to 4-(hydroxymethyl)-2-nitroacetanilide (corresponding to structure **34** in Scheme 2) which was hydrolyzed, hydrogenated, and ring-closed as described in method II to yield 5-(hydroxymethyl)-2-mercapto-1*H*-benzimidazole.

Thus, a mixture of 4-acetamidobenzoic acid (72 g, 0.4 mol), $QHSO_4$ (136 g, 0.4 mol), CH_3I (27 mL, 0.44 mol), and NaOH (32 g, 0.8 mol) in 500 mL of H_2O and 500 mL of $CHCl_3$ was allowed to react for 22 h at room temperature. The organic layer was collected, washed with 250 mL of 2.5% NaOH, dried over $MgSO_4$, and evaporated. The product was taken up in 1 L of diethyl ether and filtered to remove remaining Q salts. Evaporation of the solvent left 29 g (38%) of pure 4-acetamidobenzoic acid methyl ester.

The methyl ester (34 g, 0.18 mol) was added to 81 mL of fuming nitric acid at such a rate that the temperature could be kept below 5 °C. After completion of the addition, the mixture was poured onto ice–water. The precipitate was collected and dried *in vacuo* leaving 30 g (73%) of 4-acetamido-3-nitrobenzoic acid methyl ester.

The nitro methyl ester (13 g, 53 mmol), dissolved in 250 mL of diethyl ether and 250 mL of THF, was treated with $LiAlH_4$ (2.6 g, 69 mmol) and allowed to react for 2 h at ambient temperature. The mixture was treated with consecutive additions of 2.6 mL of H_2O, 6.9 mL of 5% NaOH, and 2.6 mL of H_2O. The solid material was filtered off and washed twice with diethyl ether. The combined organic layers were dried over $MgSO_4$, evaporated, and chromatographed on silica gel (CH_2Cl_2/MeOH, 96/4) leaving 4.6 g (41%) of fairly pure 4-(hydroxymethyl)-2-nitroacetanilide.

Step 3. Finally, reacting 5-(hydroxymethyl)-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-4-methoxy-3-methylpyridine according to method III furnished the title compound **5**: ^{1}H-NMR (MeOH-d_4) δ 2.23 (s, 3H), 3.96 (s, 3H), 4.8 (2d, partly obscured by residual protons in MeOH-d_4, 2H), 7.01 (d, 1H), 7.43 (d, 1H), 7.71 (m, 2H), 8.23 (d, 1H). Anal. ($C_{16}H_{17}N_3O_3S\cdot{}^1/_2H_2O$) C, H, N.

2-[[(4-Ethoxy-3-methyl-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (6). Step 1. The synthesis of the title compound required access to 4-ethoxy-2,3-dimethylpyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-4-ethoxy-3-methylpyridine.

Thus, 2,3-dimethylpyridine (45 g, 0.45 mol) was dissolved in 150 mL of concentrated acetic acid, mixed with 25 mL of 30% H_2O_2, and gently heated to 95 °C for 3 h. A second lot of 11 mL of 30% H_2O_2 was added and allowed to react for an additional 12 h. The solvent was evaporated[43] and the residue recrystallized from isopropyl ether leaving 44 g (80%) of 2,3-dimethylpyridine *N*-oxide.

The *N*-oxide (44 g, 0.36 mol) was dissolved in 90 mL of concentrated H_2SO_4 and treated with a mixture of 130 mL of concentrated H_2SO_4 and 150 mL of 65% HNO_3. The mixture was heated to 95 °C for 20 h. The mixture was cooled and treated with 10 M NaOH until pH 3. The precipitate was collected and crystallized from EtOH yielding 32 g (53%) 2,3-dimethyl-4-nitropyridine *N*-oxide.[44]

The nitro compound (8.8 g, 47 mmol) was dissolved in 120 mL of absolute EtOH and heated to 70 °C. NaOEt (6.4 g, 0.94 mol) was added over the next 24 h. The solvent was evaporated leaving a black tar which was used without further purification in method I.

Step 2. Finally, reacting commercially available 2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-4-ethoxy-3-methylpyridine according to method III furnished the title compound **6**: ^{1}H-NMR ($CHCl_3$-d_3) δ **1.42** (t, 3H), 2.16 (s, 3H), 4.04 (quartet, 2H), 4.72 (d, 1H), 4.82 (d, 1H), 6.69 (d, 1H), 7.30 (m, 2H), 7.63 (b, 2H), 8.29 (d, 1H). Anal. ($C_{16}H_{17}N_3O_2S\cdot{}^1/_4H_2O$) C, H, N.

5-*tert*-Butyl-2-[[(4-methoxy-3-methyl-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (7). Step 1. The synthesis of the title compound required access to 2-(chloromethyl)-4-methoxy-3-methylpyridine, *cf.* the synthesis of **5**.

Step 2. The synthesis of the title compound also required access to 4-*tert*-butylaniline which was carried through the procedure described in method II to yield 5-*tert*-butyl-2-mercapto-1*H*-benzimidazole.

Thus, a mixture of 36 mL of concentrated HNO_3 and 54 mL of concentrated H_2SO_4 was added to *tert*-butylbenzene (56 g, 0.42 mol) at such a rate that the temperature could be kept below 5 °C. After stirring for an additional hour, the mixture was poured onto ice–water. The product was extracted with diethyl ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was distilled at 0.09 Torr and the fraction coming off between 80 and 90 °C was collected: yield 54 g (72%).

The nitro compound (54 g, 0.3 mol) was dissolved in 540 mL of EtOH and hydrogenated over Pd/C. After completion of the reaction, the mixture was filtered and the solvent evaporated leaving a nearly quantitative yield of 4-*tert*-butylaniline as an oil.

Step 3. Finally, reacting 5-*tert*-butyl-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-4-methoxy-3-methylpyridine according to method III furnished the title compound **7**: ^{1}H-NMR (DMSO-d_6) δ 1.34 (s, 9H), 2.14 (s, 3H), 3.84 (s, 3H), 4.70 (d, 1H), 4.78 (d, 1H), 6.96 (d, 1H), 7.39 (dd, 1H), 7.57 (d, 2H), 8.36 (d, 1H). Anal. ($C_{19}H_{23}N_3O_2S\cdot{}^1/_2CH_3OH$)[45] C, H; N: calcd, 11.25; found, 10.8.

4-Fluoro-2-[[(4-methoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (8). The title compound was prepared according to the procedure reported in ref 46.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridyl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole (9). The title compound was prepared according to the procedure reported in ref 47.

2-[[[4-(2,2,2-Trifluoroethoxy)-3-methyl-2-pyridyl]methyl]sulfinyl]-1*H*-benzimidazole (10). The title compound was prepared according to the procedure reported in ref 48.

4,6-Difluoro-2-[[(4-methoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (11). Step 1. The synthesis of the title compound required access to 4-methoxy-2-methylpyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-4-methoxypyridine.

Thus, 2-methylpyridine (486 g, 5.22 mol) was dissolved in 1500 mL of concentrated acetic acid, mixed with 242 mL of 30% H_2O_2, and gently heated to 85 °C for 3 h. A second lot of 180 mL of 30% H_2O_2 was added (at ambient temperature) and allowed to react for an additional 12 h (at 85 °C). The reaction mixture was cooled and treated with Pd/C to destroy residual H_2O_2. After reacting for 15 min, the catalyst was filtered off and the filtrate evaporated. Toluene was used to furnish an azeotrope with residual acetic acid upon reevaporation of solvents. The residue was distilled at 18 mmHg and the

fraction coming off between 130 and 135 °C collected: yield 381 g (67%).

The *N*-oxide (44 g, 0.4 mol) was dissolved in 78 mL of concentrated H_2SO_4 and treated with a mixture of 113 mL of concentrated H_2SO_4 and 139 mL of 65% HNO_3 at 0 °C. The mixture was heated to 95 °C for 22 h. The mixture was cooled, poured onto 600 mL of ice–water, and treated with concentrated ammonia until pH 3. The aqueous layer was extracted three times with 300 mL of CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and evaporated leaving 60 g (98%) of 2-methyl-4-nitropyridine *N*-oxide[44] as yellow crystals.

The nitro compound (116 g, 0.78 mol) was dissolved in 1700 mL of MeOH and treated with 122 g of K_2CO_3 at reflux for 12 h. After cooling and filtration, the solvent was evaporated. The residue was taken up in CH_3CN, filtered, and reevaporated leaving a quantitative yield of 4-methoxy-2-methylpyridine *N*-oxide.

Step 2. The other building block in the synthesis of the title compound started from 4,6-difluoro-2-nitroaniline (corresponding to structure **33** in Scheme 2) which was hydrogenated and ring-closed as described in method II to yield 4,6-difluoro-2-mercapto-1*H*-benzimidazole.

Step 3. Finally, reacting 4,6-difluoro-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-4-methoxypyridine according to method III furnished the title compound **11**: ^{1}H-NMR ($CHCl_3$-d_3) δ 3.73 (s, 3H), 4.51 (d, 1H), 4.75 (d, 1H), 6.71 (d, 1H), 6.76 (dd, 1H), 6.86 (dt, 1H), 7.08 (bd, 1H), 8.34 (d, 1H). Anal. ($C_{14}H_{11}F_2N_3O_2S \cdot {}^1/_4H_2O$) C, H, N.

5,6-Difluoro-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazole (12). Step 1. The synthesis of the title compound required access to 3,4-dimethoxy-2-methylpyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-3,4-dimethoxypyridine.

Thus, 3-hydroxy-2-methyl-4-pyrone (13 g, 0.1 mol) and CH_3I (62 mL, 1.0 mol) were reacted over K_2CO_3 (55 g, 0.4 mol) in 500 mL of acetone for 19 h at reflux. The solvent and excess CH_3I were evaporated, and the residue was taken up in 250 mL of H_2O. The aqueous layer was extracted with 500 + 100 mL of CH_2Cl_2. The organic layer was washed with 5% Na_2CO_3, dried over $MgSO_4$, and evaporated leaving 10 g (68%) of 3-methoxy-2-methyl-4-pyrone as a brown oil.

The pyrone (8.4 g, 60 mmol) was treated with 26 mL of concentrated ammonia in a steel autoclave at 110 °C for 3 h. After cooling, the ammonia was evaporated leaving a brownish oil which partly crystallized. Trituration with acetone, filtration, and drying furnished 6.6 g (77%) of pure pyridone as beige crystals.

The pyridone (7.0 g, 50 mmol) was suspended in 62 mL of $POCl_3$ and refluxed for 10 h. The excess reagent was evaporated and the residue partitioned between 200 mL of $CHCl_3$ and 200 mL of H_2O. The aqueous layer was treated with K_2CO_3 until pH 10 and extracted with 200 mL of $CHCl_3$. The organic layers were combined, passed through phase separation papers,[49] and evaporated leaving 6.7 g (85%) of pure 4-chloro-3-methoxy-2-methylpyridine as a tan oil.

The chloropyridine (6.7 g, 43 mmol) was dissolved in 10 mL of MeOH and treated with 40 mL of 30% NaOMe (in MeOH) for 10 h at reflux. After cooling, the mixture was evaporated to dryness, treated with 50 mL of ice–water, and extracted with 200 + 100 mL of $CHCl_3$. The organic layers were combined, dried over $MgSO_4$, and evaporated yielding 5.7 g (88%) of 3,4-dimethoxy-2-methylpyridine as a brown oil.

The pyridine (3.5 g, 23 mmol) was dissolved in 140 mL of CH_2Cl_2 and treated with 84% *m*-CPBA (5.2 g, 25 mmol) at 0 °C. After reacting for 4 h at ambient temperature, the organic layer was washed with 250 mL of 5% Na_2CO_3, dried over $MgSO_4$, and evaporated leaving 3.8 g (98%) of the desired 3,4-dimethoxy-2-methylpyridine *N*-oxide.

Step 2. The other building block in the synthesis of the title compound started from 4,5-difluoro-2-nitroaniline (corresponding to **33** in Scheme 2) which was carried through the procedure described in method II (with the exception of the hydrogenation having been done over Raney Ni rather than Pd/C) furnishing 5,6-difluoro-2-mercapto-1*H*-benzimidazole.

Step 3. Finally, reacting 5,6-difluoro-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-3,4-dimethoxypyridine according to method III furnished the title compound **12**: ^{1}H-NMR ($CHCl_3$-d_3 containing 3 drops of DMSO-d_6) δ 3.86 (s, 3H), 3.92 (s, 3H), 4.68 (d, 1H), 4.76 (d, 1H), 6.84 (d, 1H), 7.46 (b, 2H), 8.19 (d, 1H). Anal. ($C_{15}H_{13}F_2N_3O_3S$) C, H, N.

2-[[[4-[(Cyclopropylmethyl)oxy]-3-methoxy-2-pyridyl]methyl]sulfinyl]-5-fluoro-1*H*-benzimidazole (13). Step 1. The synthesis of the title compound required access to 4-[(cyclopropylmethyl)oxy]-3-methoxy-2-methylpyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-4-[(cyclopropylmethyl)oxy]-3-methoxypyridine.

Thus, 3-hydroxy-2-methyl-4-pyrone (13 g, 0.1 mol) and CH_3I (62 mL, 1.0 mol) were reacted over K_2CO_3 (55 g, 0.4 mol) in 500 mL of acetone for 19 h at reflux. The solvent and excess CH_3I were evaporated, and the residue was taken up in 250 mL of H_2O. The aqueous layer was extracted with 500 + 100 mL of CH_2Cl_2. The organic layer was washed with 5% Na_2CO_3, dried over $MgSO_4$, and evaporated leaving 10 g (68%) of 3-methoxy-2-methyl-4-pyrone as a brown oil.

The pyrone (8.4 g, 60 mmol) was treated with 26 mL of concentrated ammonia in a steel autoclave at 110 °C for 3 h. After cooling, the ammonia was evaporated leaving a brownish oil which partly crystallized. Trituration with acetone, filtration, and drying furnished 6.6 g (77%) of pure pyridone as beige crystals.

The pyridone (7.0 g, 50 mmol) was suspended in 62 mL of $POCl_3$ and refluxed for 10 h. The excess reagent was evaporated and the residue partitioned between 200 mL of $CHCl_3$ and extracted with 200 mL of $CHCl_3$. The organic layers were combined, passed through phase separation papers,[49] and evaporated leaving 6.7 g (85%) of pure 4-chloro-3-methoxy-2-methylpyridine as a tan oil.

The chloropyridine (1.58 g, 10 mmol) was added to a suspension of 55% NaH in oil (1.09 g, 25 mmol) in 10 mL of cyclopropylmethyl alcohol and allowed to react for 10 h. The solvent was evaporated and the residue taken up in 100 mL of 1 M HCl. The aqueous layer was washed with 100 mL of CH_2Cl_2, treated with Na_2CO_3 until pH 10, and extracted with 100 + 50 mL of CH_2Cl_2. The latter two organic layers were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (EtOAc) furnishing 1.25 g (65%) of pure pyridine as a colorless oil.

The pyridine (1.25 g, 7 mmol) was dissolved in 40 mL of CH_2Cl_2 and treated with 48% *m*-CPBA (2.56 g, 7 mmol) at 0 °C. After reacting for 15 h at ambient temperature, the organic layer was washed with 30 mL of 5% Na_2CO_3, dried over $MgSO_4$, and evaporated leaving a quantitative yield of the desired 4-[(cyclopropylmethyl)oxy]-3-methoxy-2-methylpyridine *N*-oxide.

Step 2. The other building block in the synthesis of the title compound started from 4-fluoroaniline which was carried through the procedure described in method II (with the exception of the hydrogenation having been done over Raney Ni rather than Pd/C) yielding 5-fluoro-2-mercapto-1*H*-benzimidazole.

Step 3. Finally, reacting 5-fluoro-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-4-[(cyclopropylmethyl)oxy]-3-methoxypyridine according to method III furnished the title compound **13**: ^{1}H-NMR ($CHCl_3$-d_3) δ 0.37 (m, 2H), 0.67 (m, 2H), 1.29 (m, 1H), 3.87 (d, 2H), 3.89 (s, 3H), 4.69 (d, 1H), 4.86 (d, 1H), 6.77 (d, 1H), 7.06 (m, 1H), 7.25 (b, 1H), 7.60 (b, 1H), 8.13 (d, 1H). Anal. ($C_{18}H_{18}FN_3O_3S$) C, H, N.

5-Methoxy-2-[[[3-methyl-4-(isopropyloxy)-2-pyridyl]methyl]sulfinyl]-1*H*-benzimidazole (37). Step 1. The synthesis of the title compound required access to 2,3-dimethyl-4-(isopropyloxy)pyridine *N*-oxide which was carried through the procedure described in method I to yield 2-(chloromethyl)-3-methyl-4-(isopropyloxy)pyridine.

Thus, a steel autoclave charged with a solution of 2,3-dimethyl-4-nitropyridine *N*-oxide (9 g, 54 mmol), *cf.* the synthesis of **5**, in 55 mL of concentrated HCl was heated to 170 °C for 12 h. After cooling, the mixture was poured onto 250 mL of ice and neutralized with K_2CO_3. The aqueous layer was extracted three times with 100 mL of CH_2Cl_2. The

combined organic layers were dried over Na_2SO_4, evaporated and chromatographed on silica gel (CH_2Cl_2/MeOH, 92/8) leaving 4.5 g (53%) of 4-chloro-2,3-dimethylpyridine *N*-oxide[44] as a yellow crystalline material.

The chloro compound (2.9 g, 19 mmol) was dissolved in 40 mL of isopropyl alcohol and treated with 55% NaH in oil (0.96 g, 22 mmol) for 12 h at 80 °C.[50] After cooling, the solvent was evaporated and the residue chromatographed on silica gel (CH_2Cl_2/MeOH, 92/8) leaving 1.6 g (73%) of 2,3-dimethyl-4-(isopropyloxy)pyridine *N*-oxide.

Step 2. Reacting 2-mercapto-5-methoxy-1*H*-benzimidazole[47] with 2-(chloromethyl)-3-methyl-4-(isopropyloxy)pyridine according to method III furnished the title compound **37**: ^{1}H-NMR ($CHCl_3$-d_3) δ 1.35 (quartet, 6H), 2.15 (s, 3H), 3.85 (s, 3H), 4.58 (m, 1H), 4.65 (d, 1H), 4.78 (d, 1H), 6.68 (d, 1H), 6.93 (dd, 1H), 6.99 (b, 1H), 7.56 (b, 1H), 8.26 (d, 1H). Anal. ($C_{18}H_{21}N_3O_3S$) C, H, N.

2-[[(4-Methoxy-3-methyl-2-pyridyl)methyl]sulfinyl]-cyclopentano[1,2-*f*]benzimidazole (38). Step 1. The synthesis of the title compound required access to 2-(chloromethyl)-4-methoxy-3-methylpyridine, *cf.* the synthesis of **5**.

Step 2. The other building block in the synthesis of the title compound started from 5-aminoindane which was carried through the procedure described in method II (with the exception that the acidic hydrolysis of the acetanilide corresponding to **34** in Scheme 2 was exchanged to a basic hydrolysis with 10 M NaOH) to yield 2-mercaptocyclopentano-[1,2-*f*]benzimidazole.

Step 3. Finally, reacting 2-mercaptocyclopentano[1,2-*f*]-benzimidazole with 2-(chloromethyl)-4-methoxy-3-methylpyridine according to method III furnished the title compound **38**: ^{1}H-NMR ($CHCl_3$-d_3) δ 2.15 (m, 2H), 2.16 (s, 3H), 3.00 (m, 4H), 3.85 (s, 3H), 4.61 (d, 1H), 4.80 (d, 1H), 6.71 (d, 1H), 7.26 (b, 1H), 7.54 (b, 1H), 8.31 (d, 1H). Anal. ($C_{18}H_{19}N_3O_2S \cdot {}^3/_2H_2O$) C, H, N.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridyl)methyl]thio]-5-methoxy-1*H*-benzimidazole (39). The title compound was prepared according to the procedure reported in ref 47.

2-[[(3,5,6-Trimethyl-2-pyridyl)methyl]sulfinyl]-5-chloro-1*H*-benzimidazole (40). Step 1. The synthesis of the title compound required access to 2-(chloromethyl)-3,5,6-trimethylpyridine which was prepared as follows. A solution of 2,3,5-trimethylpyridine (100 g, 0.83 mol) dissolved in 520 mL of MeOH was added to a round-bottomed flask charged with 31 mL of concentrated H_2SO_4 and 100 mL of ice. The mixture was heated to reflux, and $(NH_4)_2S_2O_8$ (475 g, 2.1 mol) dissolved in 885 mL of H_2O and 10 M NaOH were added in such proportions that the pH was kept close to 2.5. The mixture was allowed to react for a further 2 h, after which 870 mL of MeOH was added. Solid material was filtered off and the MeOH evaporated. The remaining aqueous layer was treated with 10 M NaOH until pH 11 and extracted three times with 350 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was distilled at 0.2 mmHg and the material coming off between 94 and 98 °C collected leaving 11 g (8%) of 2-(hydroxymethyl)-3,5,6-trimethylpyridine.

The corresponding chloro compound was prepared employing $SOCl_2$ as described in method I.

Step 2. Finally, reacting commercially available 5-chloro-2-mercapto-1*H*-benzimidazole with 2-(chloromethyl)-3,5,6-trimethylpyridine according to method III furnished the title compound **40**: ^{1}H-NMR ($CHCl_3$-d_3) δ 2.22 (s, 3H), 2.26 (s, 3H), 2.30 (s, 3H), 4.60 (d, 1H), 4.78 (d, 1H), 7.21 (s, 1H), 7.28 (dd, 1H), 7.52 (b, 2H). Anal. ($C_{16}H_{16}ClN_3OS \cdot {}^1/_2H_2O$) C, H, N.

Chromatography. The retention times needed to calculate k' were determined on a Waters Novapak C18 column (4 μm spherical beads, 3.9 mm × 150 mm) at a flow rate of 0.7 mL/min employing a mobile phase of 27% CH_3CN in dilute phosphate buffer at pH 7.4. The void volume was determined by injecting sodium dichromate.

***In Vitro* Methods.** Bacteria incubated for 3 days at 37 °C under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) were gently scraped off from the Columbia blood agar plates and washed with PBS (137 mM NaCl, 5.1 mM Na_2HPO_4, 2.7 mM KCl, and 0.88 mM KH_2PO_4) adjusted to the pH which was to be used in the assay. The suspension was centrifuged at 2773*g* for 10 min at ambient temperature, and the bacteria were collected. After two additional washings, the suspension was adjusted to $A_{560} = 0.3$. The concentration of purified Jack bean urease used (18 μg/mL, 1.28 U/mL) gave the same urease activity as the bacterial suspension.

Compounds were dissolved in MeOH or DMSO and when necessary sonicated for some minutes. Aliquots were added to the test solutions to final concentrations of 1, 10, and 100 μM (with the exception of flurofamide where the concentrations used were 1, 10, and 100 nM), and the organic solvent component amounted to ≤1%. The samples were incubated for 30 min at 37 °C in a water bath with gentle shaking. The reaction was started by adding 1 part 200 mM urea solution to 1 part test solution and stopped 10 min later by adding 25 parts reagent A (10 g of phenol and 50 mg of $Na_2Fe(CN)_5NO$ dissolved in 1 L of water) and 25 parts reagent B (5 g of NaOH and 8.4 mL of NaOCl (Aldrich) dissolved in 1 L of water). The samples were incubated for a further 15 min to allow color development, after which 200 μL aliquots were transferred to 96-well microtiter plates. The absorbance at 650 nm was determined at ambient temperature using $(NH_4)_2SO_4$ as standard.

***In Vivo* Methods.** The details of the *in vivo* experiments have been described elsewhere.[28,29] In this study Methocel vehicle (0.1 mL) adjusted to pH 6 with citric acid was used and given twice daily. Drugs were given either dissolved or suspended in the vehicle, and the amounts stated are per mouse, mean body weight of 30 g, and day. Stock solutions or suspensions were stored frozen.

Triple therapy was made up by 0.185 mg of bismuth, 0.675 mg of metronidazole, and 1.500 mg of tetracycline and was administered once daily for 2 weeks followed by bismuth alone once daily for another 2 weeks. In this group of animals, vehicle alone was administered at the second daily dosing occasion. Omeprazole (1.295 mg, corresponding to 125 μmol/kg) and flurofamide (1.250 mg, corresponding to 230 μmol/kg) were each dosed twice daily for 4 weeks.

The number of animals used in the different groups was 20, 20, 30, 30, 30, and 24 in the uninfected no treatment control, the infected no treatment control, the infected vehicle control, the triple therapy group, the omeprazole group, and the flurofamide group, respectively. The suppression data are based on 10 animals each. The eradication data are based on the number of remaining animals in each group (always >10).

Acknowledgment. The corresponding author would like to thank Dr. Per Lindberg, Astra Hässle AB, for valuable and fruitful discussions at the onset of this study.

References

(1) Working Party Reports. 36–45 World Congress on Gastroenterology, Sydney, Australia, 1990.
(2) *Helicobacter pylori* in Peptic Ulcer Disease. *NIH Consens Statement* **1994** Feb 7–9, *12* (1), 1–17.
(3) Forbes, M. F.; Glaser, M. G.; Cullen, D. J. E.; Warren, J. R.; Christianssen, K. J.; Marshall, B. J. Duodenal ulcer treated with *Helicobacter pylori* eradication: seven year follow up. *Lancet* **1994**, *343*, 258–260.
(4) Labenz, J.; Rühl, G. H.; Bertrams, J.; Börsch, G. Clinical course of duodenal ulcer disease one year after omeprazole plus amoxycillin or triple therapy plus ranitidine for cure of *Helicobacter pylori* infection. *Eur. J. Gastroenterol. Hepatol.* **1994**, *6*, 293–297.
(5) Tytgat, G. N. J. Long-Term Consequences of *Helicobacter pylori* Eradication. *Scand. J. Gastroenterol. Suppl.* **1994**, in press.
(6) Bayerdörffer, E.; Ottenjahn, R. The Role of Antibiotics in *Campylobacter pylori* Associated Peptic Ulcer Disease. *Scand. J. Gastroenterol.* **1988**, *23* (Suppl. 142), 93–100.
(7) Unge, P.; Gas, A.; Eriksson, K.; Bergman, B.; Carling, L.; Ekström, P.; Glise, H.; Gnarpe, H.; Junghard, O.; Lindholmer, C.; Sandzén, B.; Strandberg, L.; Stubberöd, A.; Weywadt, L. Amoxicillin added to omeprazole prevents relapse in the treatment of duodenal ulcer patients. *Eur. J. Gastroenterol. Hepatol.* **1993**, *5*, 325–331.
(8) Logan, R. P. H.; Gummett, P. A.; Hegarty, B. T.; Walker, M. M.; Baron, J. H.; Misiewicz, J. J. Clarithromycin and omeprazole for *Helicobacter pylori*. *Lancet* **1992**, *340*, 239.

(9) Mobley, H. L. T.; Hausinger, R. P. Microbial Ureases: Significance, Regulation and Molecular Characterization. *Microbiol. Rev.* **1989**, *53*, 85–108.
(10) Ferrero, R. L.; Hazell, S. L.; Lee, A. The urease enzymes of *Campylobacter pylori* and a related bacterium. *J. Med. Microbiol.* **1988**, *27*, 33–40.
(11) Hu, L.-T.; Mobley, H. L. T. Purification and N-Terminal Analysis of Urease from *Helicobacter pylori*. *Infect. Immunol.* **1990**, *58*, 992–998.
(12) Eaton, K. Y.; Brooks, C. L.; Morgan, D. R.; Krakowka, S. Essential Role of Urease in Pathogenesis of Gastritis Induced by *Helicobacter pylori* in Gnotobiotic Piglets. *Infect. Immunol.* **1991**, *59*, 2470–2475.
(13) Ferrero, R. L.; Lee, A. The importance of urease in acid-protection for the gastric-colonising bacteria *Helicobacter-pylori* and *Helicobacter-felis* new-species. *Microbiol. Ecol. Health Dis.* **1991**, *4*, 121–134.
(14) Furr, P. M.; Taylor-Robinson, D.; Hetherington, C. M. The inhibitory effect of flurofamide on ureaplasmas and their elimination from marosets by its use. *J. Antimicrob. Chemother.* **1983**, *14*, 613–618.
(15) Andrews, R. K.; Blakeley, R. L.; Zerner, B. Urea and Urease. In *Advances in Inorganic Biochemistry*; Eichhorn, B. L., Marzilli, L. G., Eds.; Elsevier Science Publishing Co., Inc.: New York, 1984; Vol. 6, pp 245–283.
(16) Lindberg, P.; Nordberg, P.; Alminger, T.; Brändström, A.; Wallmark, B. The Mechanism of Action of the Gastric Acid Secretion Inhibitor Omeprazole. *J. Med. Chem.* **1986**, *29*, 1327–1329.
(17) Lindberg, P.; Brändström, A.; Wallmark, B. Structure-activity relationships of omeprazole analogues and their mechanism of action. *TIPS* **1987**, *8* (10), 399–402.
(18) $k' = (t_R - t_{void})/t_{void}$.
(19) We defined log k'_o as $\log(6.72k'/k'_{compound9})$.
(20) Brändström, A.; Lindberg, P.; Bergman, N.-Å.; Alminger, T.; Ankner, K.; Junggren, U.; Lamm, B.; Nordberg, P.; Erickson, M.; Grundevik, I.; Hagin, I.; Hoffmann, K.-J.; Johansson, S.; Larsson, S.; Löfberg, I.-L.; Ohlson, K.; Persson, B.; Skånberg, I.; Tekenbergs-Hjelte, L. Chemical Reactions of Omeprazole and Omeprazole Analogues. *Acta Chem. Scand.* **1989**, *6*, 536–611.
(21) References to the PLS method can be found in a recent paper: *QSAR: Chemometric Methods in Molecular Design. Methods and Principles in Medicinal Chemistry, Vol. 2*; van de Waterbeemd, H., Ed.; Verlag Chemie: Weinheim, Germany, 1994.
(22) ***m*-Chloroperbenzoic acid.**
(23) The mechanism of the rearrangement remains controversial but has been discussed; see: Paquette, A. L. In *Principles of Modern Heterocyclic Chemistry*; Breslow, R., Ed.; The Benjamin/Cummings Publishing Co., Inc.: London, 1968; Vol. 7, p 261.
(24) Ferrero, R. L.; Cussac, V.; Courcoux, P.; Labigne, A. Construction of isogenic urease negative mutants of *Helicobacter pylori* by allelic exchange. *J. Bacteriol.* **1992**, *174*, 4212–4217.
(25) Weatherburn, M. W. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* **1967**, *39*, 971–974.
(26) Kaplan, A. The determination of urea, ammonia and urease. *Methods Biochem. Anal.* **1969**, *17*, 311–324.
(27) Preliminary data disclosed; see: Lee, A.; Hegedus, E.; O'Rourke, J.; Larsson, H. Urease inhibitors not effective for treatment of helicobacter infection but do prevent colonisation. *Irish J. Med. Sci.* **1992**, *161* (Suppl. 10), 4–5 (Abstract W 2.3).
(28) Dick-Hegedus, E.; Lee, A. Use of a mouse model to examine anti-*Helicobacter pylori* agents. *Scand. J. Gastroenterol.* **1991**, *26*, 909–915.
(29) Hazell, S. L.; Borody, T. J.; Gal, A.; Lee, A. Detection of *Campylobacter pylori* as a marker of bacterial colonisation and gastritis. *Am. J. Gastroenterol.* **1987**, *82*, 292–296.
(30) Bayless, A. V.; Millner, O. E. U.S. Patent 4,182,881, 1980.
(31) The chemistry of 6-methyl derivatives is extensively reviewed in ref 20, but briefly the steric bulk introduced by the methyl group prevents the pyridine nitrogen–benzimidazole carbon bond in structure **5** to be formed; this prevents sulfenamide formation.
(32) Lindberg, P.; Brändström, A.; Wallmark, B.; Mattsson, H.; Rikner, L.; Hoffman, K.-J. Omeprazole: The First Proton Pump Inhibitor. *Med. Res. Rev.* **1990**, *10* (1), 1–54.
(33) Bugnilo, M.; Bayeli, P. F.; Rappuoli, R.; Pennatini, C.; Figura, N.; Crabtree, J. E. Inhibition of *Helicobacter pylori* urease by omeprazole. *Eur. J. Gastroenterol. Hepatol.* **1993**, *5*, 683–685.
(34) Nagata, K.; Satoh, H.; Iwahi, T.; Shimoyama, T.; Tamura, T. Potent Inhibition of the Gastric Pump Inhibitor Lansoprazole against Urease Activity of *Helicobacter pylori*: Unique Action Selective for *H. pylori* Cells. *Antimicrob. Agents Chemother.* **1993**, *37*, 769–774.
(35) Jabri, E.; Carr, M. B.; Hausinger, R. P.; Karplus, P. A. The Crystal Structure of Urease from *Klebsiella aerogenes*. *Science* **1995**, *268*, 998–1004.
(36) McColm, A. A.; Bagshaw, J.; O'Malley, C.; McLaren, A. Is urease a lethal target for therapy of *Helicobacter pylori*? *Microbial Ecol. Health Dis.* (*Proc. 6th Int. Workshop Campylobacter, Helicobacter Relat. Organisms*) **1991**, *4*, 145 (Abstract H4–10).
(37) Sjöström, J.-E.; Berglund, M.-L.; Fryklund, J.; Kühler, T.; Larsson, H. Factors affecting the antibacterial activity of omeprazole and structural analogues *in vitro*. *Acta Gastroenterol. Belg.* **1993**, *56*, 144.
(38) Great caution is advised at this instanced since these reactions are strongly exothermic and on occasion have gotten out of control.
(39) Caution was taken to avoid suction of excess air into the filtrate since it would cause massive decolorization and loss of product.
(40) On occasion the lower temperature would give a cleaner reaction.
(41) On occasion **6** was triturated with a few milliliters of EtOAc saturated with ammonia yielding a crystalline material.
(42) On occasion an oil would form and require extractive workup. Rapid isolation is expedient since the product decomposes with time.
(43) Modern synthetic methods call for *m*-CPBA to be used as oxidizing agent. Mixtures of H_2O_2 and acetic acid have **allegedly** on occasion exploded upon evaporation.
(44) The nitropyridine *N*-oxides are mutagenic, and caution is advised when handling these compounds. The same is likely to apply to the chloropyridine *N*-oxides, but this has not been investigated.
(45) This compound was purified by chromatography using an eluent containing methanol.
(46) Brändström, A. E.; Lindberg, P. L.; Sundén, G. E. U.S. Patent 5025024, 1991.
(47) Junggren, U. K.; Sjöstrand, S. E. European Patent O 005 129 A1, 1979.
(48) Nohara, A.; Maki, Y. European Patent O 174 726 B1, 1989.
(49) Catalogue no. 2200 125, Whatman Inc. Ltd., Maidstone, U.K.
(50) Exchanging part of the isopropyl alcohol with DMF allows a higher reaction temperature and gives a higher yield of the desired product.

JM950307M