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Chemistry of Covalent Inhibition of the Gastric (H^+, K^+)-ATPase by Proton Pump Inhibitors

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Abstract: Proton pump inhibitors (PPIs), drugs that are widely used for treatment of acid related diseases, are either substituted pyridylmethylsulfinyl benzimidazole or imidazopyridine derivatives. They are all prodrugs that inhibit the acid-secreting gastric (H^+, K^+)-ATPase by acid activation to reactive thiophiles that form disulfide bonds with one or more cysteines accessible from the exoplasmic surface of the enzyme. This unique acid-catalysis mechanism had been ascribed to the nucleophilicity of the pyridine ring. However, the data obtained here show that their conversion to the reactive cationic thiophilic sulfenic acid or sulfenamide depends mainly not on pyridine protonation but on a second protonation of the imidazole component that increases the electrophilicity of the C-2 position on the imidazole. This protonation results in reaction of the C-2 with the unprotonated fraction of the pyridine ring to form the reactive derivatives. The relevant PPI pK_a's were determined by UV spectroscopy of the benzimidazole or imidazopyridine sulfinylmethyl moieties at different medium pH. Synthesis of a relatively acid stable analogue, N^1-methyl lansoprazole, (**6b**), allowed direct determination of both pK_a values of this intact PPI allowing calculation of the two pK_a values for all the PPIs. These values predict their relative acid stability and thus the rate of reaction with cysteines of the active proton pump at the pH of the secreting parietal cell. The PPI accumulates in the secretory canaliculus of the parietal cell due to pyridine protonation then binds to the pump and is activated by the second protonation on the surface of the protein to allow disulfide formation.

Introduction

Inhibition of gastric acid secretion has been the major means of treatment of acid related diseases such as peptic ulcers and gastro-esophageal reflux disease. The first medicinal target to be identified was the histamine-2 receptor, the major, but not the only, activating parietal cell receptor. The second medicinal target was the gastric acid pump, the gastric (H^+, K^+)-ATPase. Since proton transport by the gastric (H^+,K^+)-ATPase is the final step in acid secretion, it was anticipated that drugs of this type would be more effective inhibitors of acid secretion.[1]

The particular properties of the gastric parietal cell allow for selective accumulation of weak bases with a pK_a < 4.0, since no other space in the body has a pH < 4.0. A compound was developed, timoprazole, pyridyl-methylsulfinyl benzimidazole, that inhibited gastric acid secretion as assessed with in vitro models such as rabbit gastric glands and in vivo, irrespective of the nature of the stimulus, indicating the compound was not a receptor antagonist (Figure 1). The pK_a1 of the compound of ~3.0 in the pyridine ring would allow selective accumulation in the acid space of the parietal cell. Experiments performed with this compound and a subsequent derivative with substitution on the pyridine (picoprazole) showed that they were able to inhibit (H^+, K^+)-ATPase activity only under acid transporting conditions. Moreover, there was a lag between administration of the drugs and the appearance of inhibition of acid secretion.[2,3] These data suggested that these weak bases were prodrugs that were acid-activated and acted as proton pump inhibitors (PPIs). Analysis of their structure suggested that the active compounds were cysteine-reactive. Experiments with the isotope-labeled drugs showed that the activation of the PPIs resulted in covalent inhibition of the enzyme solely by disulfide formation with one or more cysteine moieties present in the pump which are accessible from the luminal surface of the enzyme, since disulfide reducing agents abolished the isotope-labeling of the pump protein.[4]

Omeprazole was the first clinically useful compound of this class and was introduced in 1989.[5] Its structure, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methylsulfinyl]-*1H*-benzimidazole, is shown in Figure 1, as are the structures of the other commonly used PPIs, lansoprazole, pantoprazole, and rabeprazole which all have a benzimidazole and a newer PPI, tenatoprazole, that has an imidazopyridine ring instead of a benzimidazole. The benzimidazole PPIs are analogues of 2-(pyridinylmethylsulfinyl)benzimidazole, timoprazole.

The PPIs consist of two heterocyclic moieties. One is a pyridine moiety, and the other is a benzimidazole or an imidazo-

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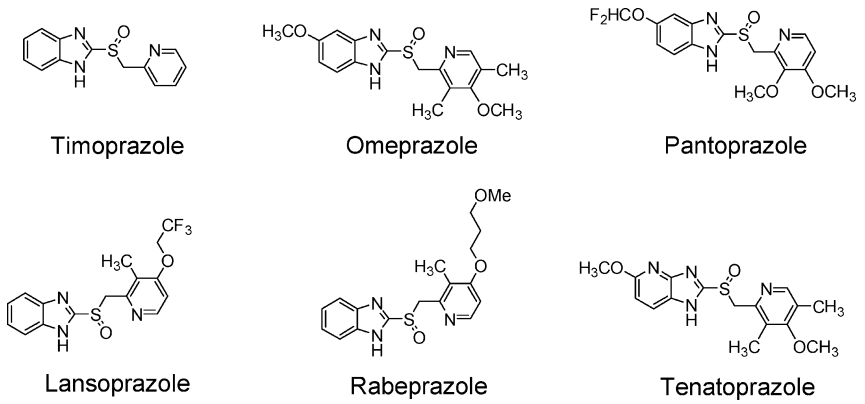

Figure 1. Structure of proton pump inhibitors. The initial PPI described (although its mechanism was not known at that time) was timoprazole which showed nonspecific effects. This was followed by picoprazole (not shown) then omeprazole, lansoprazole, pantoprazole, rabeprazole, and finally tenatoprazole.

pyridine. The two heterocyclic moieties are linked through a methylenesulfinyl ($-CH_2SO-$) group. Clinically available proton pump inhibitors are omeprazole, *S*-omeprazole (*S*-enantiomer of omeprazole), lansoprazole, pantoprazole, and rabeprazole. Lansoprazole is 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-ylmethanesulfinyl]-*1H*-benzimidazole. Pantoprazole is 5-difluoromethoxy-2-[(3,4-dimethoxy-pyridin-2-yl)methylsulfinyl]-*1H*-benzimidazole. Rabeprazole is 2-[4-(3-methoxypropoxy)-3-methyl-pyridine-2-yl]methylsulfinyl-*1H*-benzimidazole. The other type of PPI is tenatoprazole which is 5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2-yl)methylsulfinyl]-*1H*-imidazo-[4,5-*b*]pyridine, and this compound is under development.

There have been several studies examining the chemical mechanism underlying inhibition of the gastric ATPase[6] as well as defining the particular cysteines that react with the different PPIs.[4,7−9] Knowledge of the crystal structure of a similar P_2 type ATPase has also allowed development of a 3D model of the (H^+,K^+)-ATPase in which the reactive cysteines can be located.[10]

It has been shown that, under acidic conditions, omeprazole is transformed to a spiro intermediate of dihydrobenzimidazole as a result of acid catalysis which then undergoes aromatization to a sulfenic acid followed by dehydration to a tetracyclic sulfenamide.[6] Both are permanent cations and react with thiol groups to form chemically stable disulfides.

The stability of the different PPIs has been measured at a pH of 5.1. The stability of the PPIs measured as their $t_{1/2}$ was as follows: pantoprazole 4.7 h, lansoprazole 1.5 h, omeprazole 1.4 h, and rabeprazole 0.12 h.[11] At a pH of 1.2 which is nearer to the prevailing pH of the acid space of the parietal cell, the PPIs were activated much more rapidly: their half-lives ranged from 4.6 to 1.3 min, the slowest being pantoprazole. This report did not explain why PPIs were more rapidly activated at pH 1.2 than at pH 5.1.[11] Generally, it was well accepted that the substituents on the pyridine ring influence the rate constants of conversion to the active form. A high pK_a of the pyridine appears relatively unstable, and it was concluded that the formation of a positive charge on the pyridine was the rate-limiting step.[12−14] In contrast, our data suggest that protonation of the benzimidazole or imidazopyridine moiety plays a decisive role in the activation of these compounds to reactive thiophiles. We conclude that the relative amounts of the protonated benzimidazole or imidazopyridine, i.e., the electrophilicity of the C-2 carbon and the proportion of the unprotonated, thus reactive, nucleophilic pyridine ring, determines the rate of activation of this class of drug. The reaction rate is thus influenced by the protonation of the benzimidazole or imidazopyridine and the nucleophilicity of the unprotonated pyridine. The rate of conversion may also determine access of some of these compounds to cysteine 822, above their common binding region in the vicinity of cysteine 813, in the luminal vestibule of the pump. With rapid conversion to the thiophilic intermediate, there is rapid reaction with cysteine 813, which prevents access to cysteine 822. With slow conversion, cysteine 822 can also be accessed by the protonated compounds prior to activation. This hypothesis explains the relatively selective reactivity of pantoprazole with cysteine 822 as compared to the other clinically available PPIs.

Methods of pK_a Measurement. The stability of the PPIs at various pH values was determined by measuring the amounts of PPI that remained at different times in the absence of thiol compounds. In acid, the PPIs convert to active forms, a sulfenic acid and a cyclo-sulfenamide by acid-catalyzed rearrangement. Active forms of the sulfenic acids and sulfenamides self-react and generate many other compounds which cannot be quantitated.[15] However, by measuring the residual PPI concentration at a given pH, the conversion rate can be obtained. The rate of disappearance of PPI was therefore measured to determine the conversion rate.

The actual pK_a1 (pyridine protonation) of the PPIs was measured by a UV spectroscopic method.[16] UV scanning was done within 25 s with fresh sample preparations at a given pH

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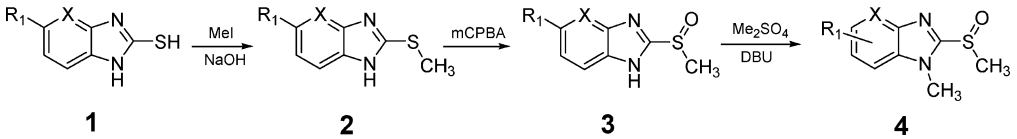
Scheme 1. Synthesis of 2-Methylsulfinyl Benzimidazoles (Compounds **3** and **4**) and N^1-Methyl Proton Pump Inhibitors (Compounds **6**)



1 **2** **3** **4**

3a: R_1=H, X=CH;
3b: R_1=OCH_3, X=CH;
3c: R_1=$OCHF_2$, X=CH;
3d: R_1=OCH_3, X=N

4a: R_1=H, X=CH;
4b: R_1=OCH_3, X=CH;
4c: R_1=$OCHF_2$, X=CH;
4d: R_1=OCH_3, X=N

5 **6**

5a: R_1=R_3=OCH_3, R_2=R_4=CH_3, X=CH ;
5b: R_1=R_4=H, R_3=OCH_2CF_3, R_2=CH_3, X=CH ;
5c: R_1=R_4=H, R_3=$OCH_2CH_2CH_2OCH_3$, R_2=CH_3, X=CH ;
5d: R_1=$OCHF_2$, R_3=OCH_3, R_2=OCH_3, R_4=H, X=CH ;
5e: R_1=R_3=OCH_3, R_2=R_4=CH_3, X=N ;

6a: R_1=5 and 6-OCH3, R_3=OCH_3, R_2=R_4=CH_3, X=CH ;
6b: R_1=R_4=H, R_3=OCH_2CF_3, R_2=CH_3, X=CH ;
6c: R_1=R_4=H, R_3=$OCH_2CH_2CH_2OCH_3$, R_2=CH_3, X=CH ;
6d: R_1=5 and 6-$OCHF_2$, R_3=OCH_3, R_2=OCH_3, R_4=H, X=CH ;
6e: R_1=5 and 6-OCH3, R_3=OCH_3, R_2=R_4=CH_3, X=N ;

Table 1. Half-Lives (min) of Proton Pump Inhibitors at 25 °C

pH	omeprazole	lansoprazole	pantoprazole	tenatoprazole
−0.301	2.9	2.2	4.6	1.6
0	3.6	1.4	3.4	2.0
0.301	4.5	1.4	4.7	3.0
0.602	3.2	1.5	5.8	4.7
0.824	3.8	1.9	6.9	8.2
1.3	4.7	3.2	9.3	13
2.3	7.3	5.3	13	26
3.1	9.0	5.9	15.5	30
4.1	13	11	41	52
5.1	68	54	265	128
6.1	462	385	1947	223
7.1	2310	1619	6477	346
7.4	nd[a]	nd	nd	533
8.0	nd	nd	nd	1386

[a] nd indicates that the half-life was not determined at some pH values.

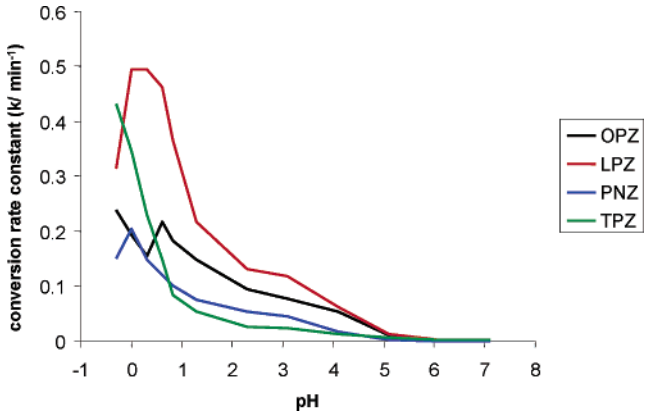


Figure 2. Conversion rate constants of proton pump inhibitors at different medium pH values. OPZ, LPZ, PNZ, and TPZ represent omeprazole, lansoprazole, pantoprazole, and tenatoprazole, respectively. The increase as a function of acidic pH and then the decrease at highly acidic pH should be noted. The first phase is due to imidazole protonation and reaction with the unprotonated pyridine, and the second phase is due to a decrease of the unprotonated pyridine at highly acidic pH.

in order to minimize the production of "active forms" generated by acid at pH ≥ 2.5. This provided the pK_a of the protonation of the pyridine moiety (pK_a1). There were characteristic changes of absorbance at different wavelengths, and the stabilization of these changes at the different pH values allowed calculation of the pK_a's of the pyridine moieties. The wavelength chosen for measurement of the pyridine pK_a1 was 250.5 or 281.5 nm, and 301 nm gave essentially identical results. Characteristically the spectral changes were observed only over the pH range close to the appropriate pK_a1.

To allow measurement of the pK_a1 and pK_a2 of the PPIs themselves, the relevant acid stable 2-methylsulfinyl benzimidazoles (**3a**−**3c**) or 2-methylsulfinyl imidazo[4,5-*b*]pyridine (**3d**) without the pyridine linked via a methylsulfinyl bridge were synthesized and their pK_a values were measured. To measure both pK_a values in a single entity, the more acid stable N^1-methyl PPIs were synthesized. The measured pK_a1 and pK_a2 for N^1-methyl lansoprazole (**6b**) allowed calculation of the relevant pK_a's of the other PPIs by providing a correction factor for the benzimidazole or imidazo-pyridine sulfinylmethyl pK_a's when the pyridine was attached.

Synthesis of the Compounds. The various steps of synthesis are illustrated in Scheme 1. The first three reactions allow formation of the relevant benzimidazoles or imidazopyridines (X═N). Methylation of compounds **5** provides the more acid stable N^1-methyl PPIs(**6**). These methods are generally applicable to all of the known PPI derivatives and produce virtually quantitative levels of the final desired products as analyzed by NMR and high-resolution mass spectroscopy. UV spectroscopy of the products of compounds **3** and compounds **6** were important in the final estimation of the pK_a's.

Results

Stability of Proton Pump Inhibitors. The conversion rates of PPIs by acid catalysis in the absence of thiol compounds occurred according to first-order kinetics. The half-lives of PPIs are summarized in Table 1, and the conversion rate constants are shown in Figure 2. The conversion rate of rabeprazole was

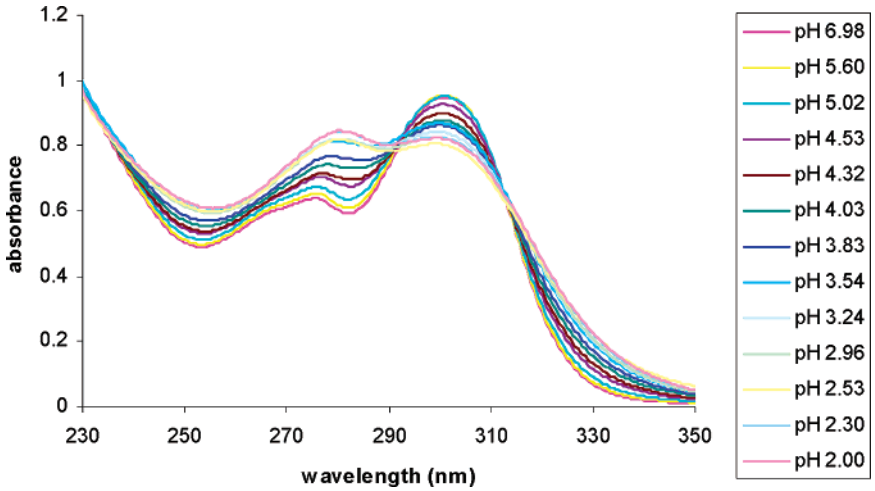


Figure 3. UV spectra of omeprazole in media of different pH. Omeprazole was scanned at different fixed medium pH values. The spectrum obtained at pH 6.98 reflects the nonionized species of omeprazole. The spectrum changes maximally at 250.5, 281.5, and 301 nm as the pH falls to pH ~3.0. The spectra obtained at lower pH values remain identical which suggests that the changes observed are due only to formation of the monoprotonated species of omeprazole and no effect is seen due to the second protonation. The spectrum obtained at pH 2 shows initial effects of the diprotonated species of omeprazole but does not allow calculation of pK_a2. However, the optical density of mono-ionized species can be calculated as described in the Experimental Section. Based on these spectra, analysis at 250.5 nm gives a pK_a1 of 4.058, and the calculated pK_a1 values were 4.08 and 4.07 at 281.5 and 301 nm, respectively. Since the absorbances at 281.5 and 301 nm were somewhat influenced by the second protonation, absorbances of monoprotonated species at these wavelengths would need to be adjusted by subtracting the effect of diprotonated species from observed absorbance. Hence 250.5 nm was chosen as the best for the determination of the pK_a1 of omeprazole.

measured at only two pH values because of a greater instability at near neutral pH and is not included in Table 1. The range examined is in general between pH 7.1 and −0.3 which encompasses both pK_a values.

As can be seen, omeprazole, lansoprazole, and pantoprazole were relatively stable until the external pH fell to 5.1 (Table 1 and Figure 2). Below that pH, conversion accelerated until the pH reached ~1.0. Pantoprazole showed a slower conversion rate at all pH values. Tenatoprazole, however, showed a five- to six-fold decrease in stability at pH 7.1 compared to the other PPIs, but its conversion at acidic pH was even slower than that of pantoprazole and its conversion rate accelerated below pH 0.6. All the PPIs showed a biphasic rate, slowing at highly acidic pH values due to the depletion of the unprotonated pyridine species that can react with the electrophilic C-2 carbon of benzimidazole ring.

The UV spectrum obtained by fast scanning between pH 2 to pH 7 was stable enough to calculate the pK_a1. A typical UV spectrum of omeprazole at various levels of pH is shown in Figure 3. With this method the pK_a1 values are 4.06 for omeprazole, 3.83 for lansoprazole, and 3.83 for pantoprazole.

pK_a Values for Benzimidazoles or Imidazo[4,5-*b*]pyridine. Rapid formation of the reactive forms of the PPIs could not be avoided when UV scanning was carried out below pH 2, a pH which is essential for determination of the relevant benzimidazole pK_a2. Since direct measurement of protonation of the benzimidazole ring pK_a2 of the PPIs is prevented by their acid instability, we synthesized acid-stable derivatives of 2-methylsulfinyl benzimidazoles (**3a**−**3c**) or imidazo[4,5-*b*]pyridine (**3d**), which are the structures where a pyridine moiety of the PPIs was removed. Also, we synthesized *N*-methyl-2-methylsulfinyl benzimidazoles (**4**) in order to define the effect of *N*-methylation on the pK_a of these structures. These derivatives are more stable at acidic pH, so we could determine their pK_a's (Table 2).

N-Methylation of 2-methylsulfinyl benzimidazole increased the pK_a by 0.08 units from the pK_a of 2-methylsulfinyl benzimidazole. When an electron-donating substituent, such as a methoxy group, is attached, the pK_a was increased by 0.1 units. In the presence of an electron-withdrawing substituent such as difluoromethoxy group, a small rise of 0.03 units was obtained after *N*-methylation.

Table 2. pK_a's of 2-Methylsulfinyl-*1H*-benzimidazoles (**3**) and 1-Methyl-2-methylsulfinylbenzimidazoles as Determined by UV Spectroscopy at Different pH Values (**4**)

compounds	pK_a of benzimidazole (analytical wavelength)
3a	1.35 ± 0.03 (255 or 281 nm)
3b	1.52 ± 0.07 (260 nm)
3c	0.84 ± 0.01 (256 or 287 nm)
3d	0.61 ± 0.05 (239 nm)
4a	1.43 ± 0.24 (281 nm)
4b[a]	1.62 ± 0.10 (259.5 nm)
4c[a]	0.87 ± 0.12 (289.5 nm)

[a] 1:1 mixture of 5- and 6-geometric isomers.

Table 3. Half-Lives (min) of N^1-methyl PPIs at Acidic pH Values[a]

pH	N-Me OPZ	N-Me LPZ	N-Me PNZ	N-Me TPZ
2	80	144.1	266.5	433.1
1.6	23.6	67.5	231	408
1.3	16.8	42.6	47.8	56.8
0.8	8.9	22.3	28.1	32.8
0.6	10.5	18.4	16.8	18.3
0.3	16.3	28.5	17.5	9.5
0	48.5	114.4	21.1	9.6
−0.3	231	140.4	79.7	16.5

[a] N-Me OPZ, N^1-methyl omeprazole (1:1 mixture of 5-methoxy and 6-methoxy isomers); N-Me LPZ, N^1-methyl lansoprazole; N-Me PNZ, N^1-methyl pantoprazole (1:1 mixture of 5-difluoromethoxy and 6-difluoromethoxy isomers); N-Me TPZ, N^1-methyl tenatoprazole (1:1 mixture of 5-methoxy and 6-methoxy isomers).

Stability of N^1-Methyl Proton Pump Inhibitors. To attempt a more accurate determination of both the pK_a's of the PPIs, we synthesized N^1-methyl derivatives of the PPIs which were stable below pH 2, as summarized in Table 3. At pH 2, the acid stabilities of N^1-methyl pantoprazole and N^1-methyl ten-

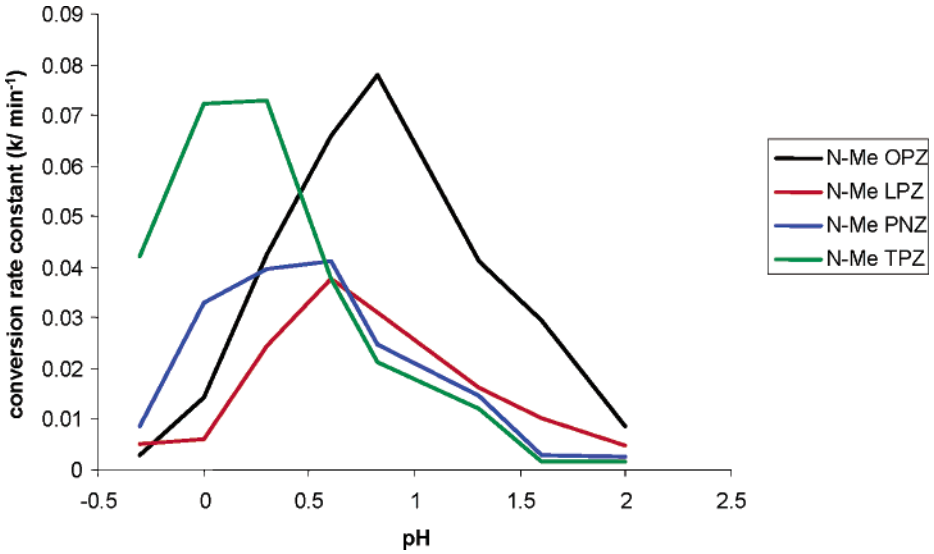


Figure 4. Conversion rate constants of N^1-methyl PPIs at different pH values. N-Me OPZ, N-Me LPZ, N-Me PNZ, and N-Me TPZ represent N^1-methyl omeprazole, N^1-methyl lansoprazole, N^1-methyl pantoprazole, and N^1-methyl tenatoprazole, respectively. It is noteworthy that at highly acidic pH the rate constant of conversion for these compounds decreases due to the persistence of the nonreacting diprotonated species as might happen in the fully active parietal cell.

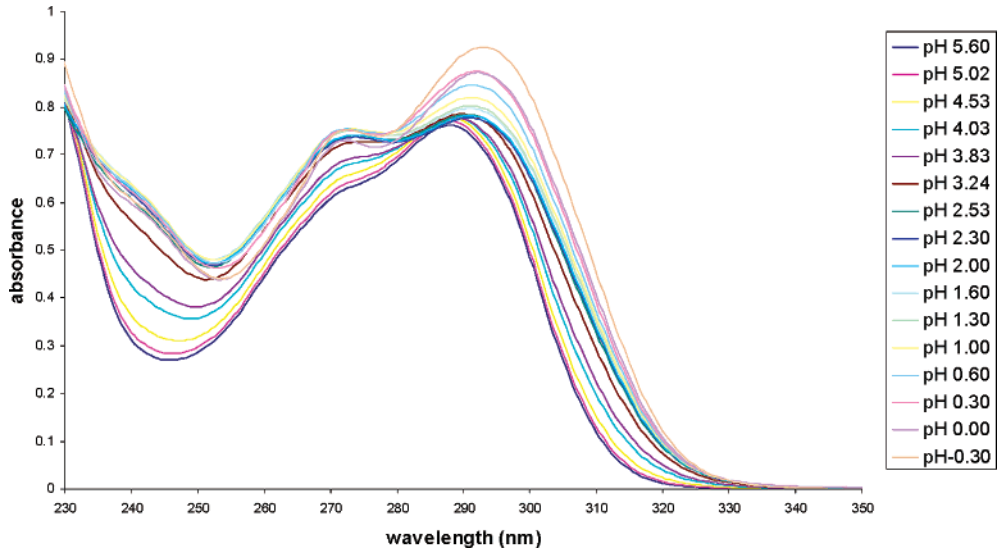


Figure 5. UV spectra of N^1-methyl lansoprazole (**6b**). The spectrum obtained at pH 7 was identical with the spectrum obtained at pH 5.6, which is that of the nonionized species. The spectra obtained between pH 2.53 and pH 2 were very similar, which suggests that this is the spectrum of the monoprotonated species, with protonation at the pyridine of N^1-methyl lansoprazole and this absorbance changes as a function of pH allowing calculation of this first pK_a1.

atoprazole was greater than those of the same derivatives of omeprazole and lansoprazole. However, N^1-methyl lansoprazole was more stable between pH 0.6 and 0, which enabled measurement of the pK_a2. The conversion rate of N^1-methyl lansoprazole was slower as compared to the unmodified PPIs (Figure 4).

pK_a's of N^1-Methyl Lansoprazole. N^1-Methyl lansoprazole (**6b**) is expected to have two pK_a values. pK_a1 is the pK_a of monoprotonated species, mainly, the pyridine pK_a, and pK_a2 is the pK_a of diprotonated species, mainly, N-3 of benzimidazole pK_a.

The spectrum of **6b** over the pH range used here showed two different absorption peaks (Figure 5). One absorption peak was largely dependent on the first protonation, that of the pyridine moiety (Figure 6A), and the other was dependent on the second protonation, that of the benzimidazole ring (Figure 6B). The absorbance of **6b** measured at 246 nm showed almost no change with a second protonation occurring between pH 1.6 and pH −0.3, but a large shift with the first protonation was found between pH 4.5 to pH 2.53. Hence the pK_a1 was calculated by measurement of change of absorbance at this 246 nm wavelength. Since the absorbance at 288−291 nm showed a very small change until the pH was less than 2.3, but a large change below that pH due to the second protonation, the pK_a2 was measured by absorbance changes at 291 nm (Figures 5 and 6B). Hence these two wavelengths, 246 and 291 nm, were chosen to measure absorbance over the full range of pH. From these analyses at the two wavelengths, the pK_a1 of N^1-methyl lansoprazole was calculated to be 3.74 ± 0.06 and the pK_a2 of N^1-methyl lansoprazole was approximately 0.7.

This method allowed measurement of the pK_a1 of the N^1-methyl PPIs (**6**) but the absorbance of the other N^1-methyl derivatives, except for that of N^1-methyl lansoprazole at 291 nm, was too broad to allow accurate measurement of pK_a2.

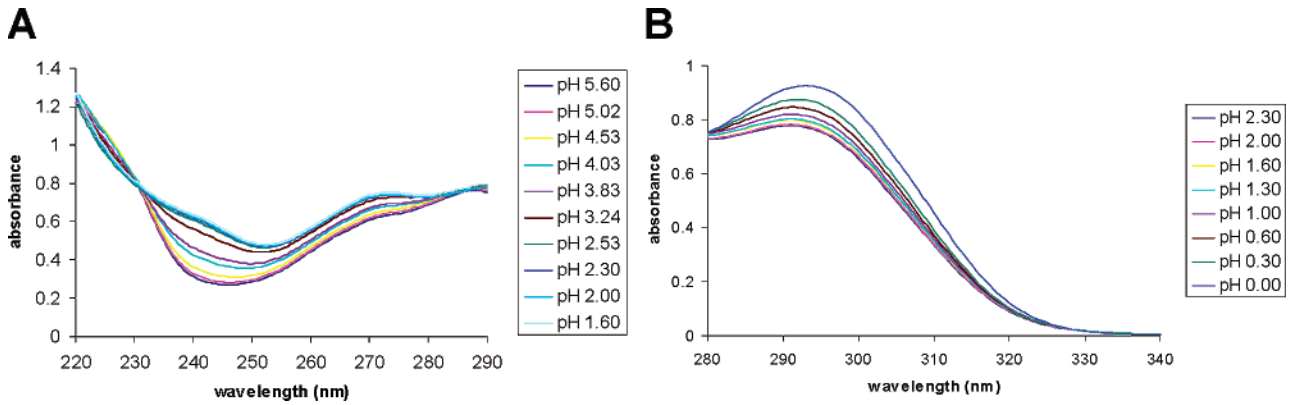


Figure 6. UV spectra near the pK_a1 (A) and near the pK_a2 (B) of N^1-methyl lansoprazole (**6b**). (A) The optical density at 246 nm was changed significantly depending on the amounts of mono-ionized species, which could then be used for determining the pK_a1 of all the PPIs. (B) Optical density at 291 nm changed significantly depending on the amounts of diprotonated species, which could then be used for determining the pK_a2 of this compound.

Table 4. pK_a's of N^1-methyl Proton Pump Inhibitors

compounds	pK_a1 (analytical wavelength)	pK_a2
N^1-methyl omeprazole (**6a**)	3.76 ± 0.17 (251 nm)	
N^1-methyl lansoprazole (**6b**)	3.74 ± 0.06 (246 nm)	~0.7 (291 nm)
N^1-methyl rabeprazole (**6c**)	4.44 ± 0.15 (247.5 nm)	
N^1-methyl pantoprazole (**6d**)	3.74 ± 0.19 (247.5 nm)	
N^1-methyl tenatoprazole (**6e**)	3.74 ± 0.12 (251 nm)	

Hence this approach allowed only both pK_a's of the N^1-methyl lansoprazole to be determined. The other pK_a1s of N^1-methyl PPIs were determined and summarized in Table 4.

Discussion

Stability and pK_a of Proton Pump Inhibitors. Proton pump inhibitors are easily activated under acidic pH conditions, converting into the thiol-reactive sulfenic acid or cyclosulfenamide through intramolecular rearrangement.[6,17,18] The conversion rates of PPIs by acid catalysis occurred according to first-order kinetics.[13] It is difficult to perform a precise acid titration curve to determine the two pK_a's of the PPIs, since PPIs are rapidly converted into their "active form" under acidic condition. Therefore, fast pH titration in acid had been carried out previously to reduce the amounts of "active form" to measure pK_a. Also, due to poor solubility of PPIs in aqueous solution, addition of methanol was necessary to improve the PPI solubility.[14] The methanol solvent effect on the pK_a had also to be taken into account.[19] The reported pK_a1 values of the PPIs were as follows: omeprazole, 3.98 [13,14] or 4.13;[20] pantoprazole, 3.92 [18] or 3.96;[20] and lansoprazole, 4.01.[20] The second pK_a was not measured but calculated.[13,14]

Here, UV spectral analysis was used to measure the pK_a's of the different compounds. The advantages of this method is that UV scanning can be done more rapidly than pH titration and requires a much lower concentration of substance measured enabling avoidance of the use of methanol. The pK_a1 values of omeprazole, lansoprazole, and pantoprazole, obtained with this technique were similar to values reported in the literature. However, rabeprazole was not stable enough to allow the measurement of the absorbance of the unchanged structure. Therefore the pK_a1 of rabeprazole could not be measured either by a fast titration method or by UV spectroscopy.

The pK_a1 of the N^1-methyl derivatives of PPI, which are relatively stable under acidic condition, were measured, and then the pK_a1 of the actual PPIs was deduced by determining the effect of *N*-methylation. The stability of N^1-methyl PPIs (**6**) below pH 2 increased significantly as shown in Table 3, and we could measure the pK_a1 of N^1-methyl PPIs as shown in Table 4.

The N^1-methyl group is far from the pyridine moiety, so the effect of a N^1-methyl group on pyridine pK_a was expected to be very small. As a result, the pyridine pK_a1's of a N^1-methyl PPI and an unmodified PPI are expected to be very similar. As expected, there were small differences between N^1-methyl PPIs and the PPIs. The effect of the N^1-methyl group on the pyridine pK_a1 can be deduced by comparing the pK_a1 of PPIs with the pK_a1 of N^1-methyl PPIs, when the pK_a1 of PPIs and the pK_a1 of N^1-methyl PPIs are available. Hence, we could calculate the pK_a1 of pyridine of the acid-labile PPIs from the measured pK_a1 of N^1-methyl PPIs. N^1-Methyl rabeprazole (**6c**) is stable enough to allow UV scanning to obtain its pK_a1. We could observe the difference of the pK_a1's between lansoprazole (**5b**) and N^1-methyl lansoprazole (**6b**), so we can approximate the pK_a1 of rabeprazole (**5c**) from the pK_a1 of N^1-methyl rabeprazole (**6c**), since the benzimidazole ring moiety is identical for rabeprazole and lansoprazole, so the *N*-methylation effect will be the same for both lansoprazole and rabeprazole.

N^1-methylation of the unsubstituted benzimidazole ring reduced the pK_a1 of the pyridine by −0.09 unit, which was derived from the measured pK_a1 data of N^1-methyl lansoprazole (**6b**) and lansoprazole (**5b**). The pK_a1 of N^1-methyl rabeprazole was measured as 4.44. Therefore, we could calculate the rabeprazole pK_a1 to be 4.53 by subtracting the *N*-methyl group effect, i.e., −0.09. The pyridine moiety of rabeprazole has the electron-donating 4′-methoxypropoxy substituent, which seems to increase the pK_a1 compared to other PPIs. The N^1-methyl tenatoprazole (**6e**) pK_a1 was measured as being 3.74. The

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pyridine moiety of tenatoprazole (**5e**) is identical to that of omeprazole (**5a**), and the N^1-methyl omeprazole (**6a**) pK_a1 was 3.76. Therefore, the imidazopyridine ring effect on the pK_a1 will decrease the pK_a by −0.02 units as compared to the benzimidazole ring of omeprazole. So, the tenatoprazole pK_a1 can be calculated by subtracting the imidazopyridine effect from the omeprazole pK_a1, 4.06. After 0.02 was subtracted due to the imidazopyridine effect from omeprazole pK_a1, the tenatoprazole pK_a1 was predicted to be 4.04, very close to that of omeprazole. Tenatoprazole has another pyrido moiety at the imidazopyridine ring, and the effect of the two nitrogens in this region is to decrease the pK_a2 at the essential C-2 position, accounting for the finding that this compound has the lowest activating pK_a.

The PPIs have very similar substituents (alkoxy and alkyl group) on the pyridine moiety with their pK_a1 ranging between 3.8 and 4.5. Below pH 2, most of the pyridine moiety of PPIs will be protonated. This protonated pyridine moiety is linked to benzimidazole through a methylenesulfinyl group (−CH_2-SO−). Different substituents on the pyridine ring would not result in any significant differential effect on the pK_a2 of the benzimidazoles or imidazopyridines of the PPIs, since these substituents are far from the benzimidazole ring and because the effect must be delivered through the covalent methylenesulfinyl bridge between the pyridine and the benzimidazole or imidazo-pyridine.

Assuming that the benzimidazole pK_a's of N^1-methyl lansoprazole (**6b**), 2-methylsulfinyl-1*H*-benzimidazole (**3a**), and 1-methyl-2-(methylsulfinyl)benzimidazole (**4a**) are 0.7, 1.35, and 1.43, respectively , as measured, pyridinylmethylsulfinyl substituents decrease the pK_a by −0.73 units compared to the pK_a of the 2-methylsulfinyl substituent and *N*-methylation increases the pK_a by +0.08 on benzimidazole. Hence, the pK_a2 of lansoprazole will be approximately 0.62, calculated by subtracting the *N*-methylation effect from N^1-methyl lansoprazole or by adding pyridinylmethylsulfinyl effect to 2-methylsulfinyl-1*H*-benzimidazole. With the hypothesis that the effect of the protonated pyridinylmethylsulfinyl group is the same among the different PPIs for the pK_a2's, then the omeprazole pK_a2 can be calculated by adding the pyridinylmethylsulfinyl effect to 5-methoxy-2-methylsulfinyl-1*H*-benzimidazole which had a pK_a of 1.52. For N^1-methyl lansoprazole (**6b)** and 1-methyl-2-(methylsulfinyl)benzimidazole (**4a**), the pK_a2's measured are 0.7 and 1.43, respectively, which shows that the pyridinylmethylsulfinyl substituent decreases ΔpK_a by 0.73 unit. So, we can calculate the pK_a2 of omeprazole (**5a**) as being 0.79 after subtracting the pyridylmethylsulfinyl effect ΔpK_a −0.73 from the 5-methoxy-2-methylsulfinyl-1*H*-benzimidazole pK_a 1.52. Similarly, the pK_a2 of pantoprazole and rabeprazole and the pK_a2′ of the imidazole moiety of tenatoprazole can be estimated. These data are summarized in Table 5.

Conversion Rates and pK_a's. The PPIs are composed of a benzimidazole or imidazopyridine ring linked via a methylenesulfinyl bridge to a substituted pyridine moiety. The conversion rate of PPIs to the active sulfenic acid or cyclo-sulfenamide is determined by both electrophilicity of C-2 of the benzimidazole ring and nucleophilicity of the pyridine moiety. The electrophilic attack by the C-2 of the protonated imidazole on the nucleophilic pyridine N enables an intramolecular rearrangement generating a C(2)−N(pyridine) bond to form the thiophilic sulfenic acid and after dehydration the cyclo-sulfenamide, both of which are permanent cations and membrane impermeant. These can then bind the luminally exposed cysteines of the (H^+,K^+)-ATPase and do not cross the membrane of the secretory canaliculus, the location of the activated ATPase

Table 5. pK_a's of Proton Pump Inhibitors

compounds	pK_a1 (analytical wavelength)	estimated pK_a2
omeprazole	**4.06 ± 0.25 (250.5 nm)**[a]	0.79[b]
lansoprazole	**3.83 ± 0.15 (240.5 nm)**[a]	0.62[b]
pantoprazole	**3.83 ± 0.24 (247.5 nm)**[a]	0.11[b]
rabeprazole	4.53[c]	0.62[b]
tenatoprazole	4.04[d]	−0.12[e]

[a] Bold shows actual measurements, and others are calculated. [b] pK_a2 was estimated by adding the "protonated pyridinylmethylsulfinyl effect" −0.73 to the 2-methylsulfinyl-1*H*-benzimidazole pK_a. [c] The pK_a1 was calculated by subtracting the *N*-methylation effect (calculated from lansoprazole pK_a1 and N^1-methyl lansoprazole pK_a1) from the N^1-methyl rabeprazole pK_a1. [d] The pK_a1 of tenatoprazole was calculated by subtracting imidazopyridine effect from omeprazole pK_a1. The imidazopyridine effect on pK_a was calculated from N^1-methyl omeprazole and N^1-methyl tenatoprazole. [e] The pK_a of the imidazo moiety of tenatoprazole was estimated by adding the "protonated" pyridinylmethylsulfinyl −0.73 to the pK_a of 2-methylsulfinyl-5-methoxy-1*H*-imidazo[4,5-*b*]pyridine.

The nucleophilicity of the pyridine ring depends on the electronic effect of substituents on the pK_a of the pyridine ring. When electron-donating substituents are attached to the pyridine moiety, the pK_a of pyridine increases, thus increasing its protonation at any given pH. Protonation of the pyridine ring then obviously decreases its nucleophilic reactivity due to the occupation of the lone pair of electrons of the pyridine N by the proton. However, the nucleophilic reactivity of the unprotonated form is increased. Substituents on the benzimidazole influence the electron density at the C-2 position. Although electron-donating substituents will decrease the electrophilicity of the C-2 position of benzimidazole, they will raise its pK_a2 hence increasing protonation on N-3 position of benzimidazole at a given pH. The converse is true of electron-withdrawing substituents where protonation on the N-3 position of benzimidazole will be decreased at a given pH. Protonation of the benzimidazole N-3 position increases the electrophilicity of the C-2 carbon, since protonation of the vicinal nitrogen will withdraw electrons from the C-2 position. This will dramatically increase the rate of reaction with the fraction of the pyridine N that is not protonated.

The steps of the major reaction pathway for activation of the PPIs are illustrated in Figure 7. First the pyridine ring is protonated with a pK_a of ∼4.0 allowing selective accumulation in the acid space of the secreting parietal cell. Next, the benzimidazole or imidazo-pyridine is protonated with a pK_a ≤ 1.0; this occurs at a rapid rate in the acidic space of the parietal cell or on the surface of the ATPase (pH ∼0.8). The fraction of the unprotonated pyridine that is present at any given pH then forms the sulfenic acid by reaction with the electrophilic C-2 carbon of the protonated imidazole moiety to form the sulfenic acid. Dehydration forms the sulfenamide. Either species can react with the luminally accessible cysteines of the gastric (H^+,K^+)-ATPase bordering on the acid space of the parietal cell, to form chemically stable enzyme disulfide derivatives.

Justification of Major Pathway. We propose three forms of the PPIs after protonation, which are Bz−PyH^+, BzH^+−Py,

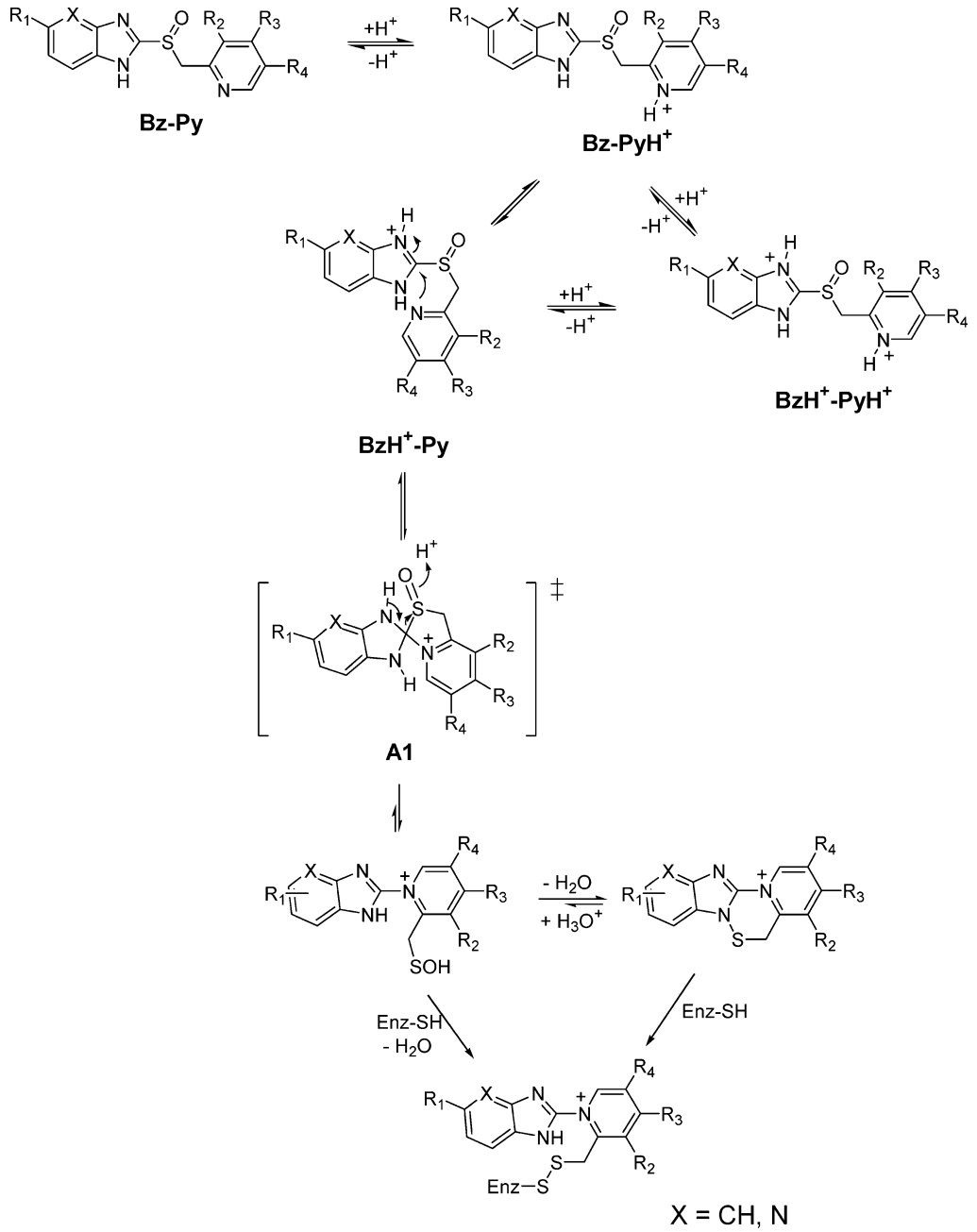

Figure 7. Mechanism of acid activation of proton pump inhibitors. The first step is protonation of the pyridine nitrogen in all the PPIs. This accounts for the accumulation in the parietal cell. The second protonation is the activation step. In all the PPIs, the activating protonation occurs at the nitrogen vicinal to the C-2 position of the imidazole ring. In the particular case of tenatoprazole where X is N instead of C as in all other PPIs, the second protonation is spread throughout the imidazopyridine. This second protonation results in a fraction of the species being present with an activated C-2 position and an unprotonated pyridine, which can then proceed to form the sulfenic acid via a transition state $[A1]^{\ddagger}$. In solution, this proceeds to form the sulfenamide by dehydration. In the presence of thiols the sulfenic acid reacts to form the disulfide, hence accelerating the reaction. Either the sulfenic acid or the sulfenamide can react with the luminally accessible cysteines of the gastric (H^+,K^+)-ATPase.

and BzH^+-PyH^+ as shown in Figure 7. It is not easy to measure the individual concentrations of $[Bz-PyH^+]$, $[BzH^+-Py]$, $[BzH^+-PyH^+]$, and $[Bz-Py]$ at a given pH, since the intramolecular reaction of the free pyridine with the electrophilic C-2 position will be very fast once the C-2 position becomes sufficiently electrophilic following N-protonation on benzimidazole. Therefore, the formation rate of BzH^+-Py or $[BzH^+-Py]$ will be the dominant factor determining the conversion rate under physiological pH ranges, the highly acidic secretory canaliculus of the active parietal cell.

The amounts of $Bz\text{-}Py{\cdot}H^+$ at given pH can be calculated from measured pK_a1 as follows.

$$PPI{\cdot}H^+ \xrightarrow{K_a1} PPI + H^+$$

$$K_a1 = \frac{[PPI][H^+]}{[PPI{\cdot}H^+]}$$

PPI has a benzimidazole moiety and a pyridine moiety, Bz−Py. So, we may rewrite as follows.

$$K_a1 = \frac{[Bz-Py][H^+]}{[(Bz-Py){\cdot}H^+]}$$

$(Bz-Py){\cdot}H^+$ is composed of two forms, $Bz-Py{\cdot}H^+$ and $Bz{\cdot}H^+-Py$. However, $[Bz{\cdot}H^+-Py]$ is very small, since Bz·

H^+ is related with pK_a2 which is 3 pK_a units lower than pK_a1. So, we may ignore [Bz·H^+−Py] and can rewrite as follows.

$$K_a1 = \frac{[\text{Bz}-\text{Py}][\text{H}^+]}{[\text{Bz}-\text{Py}\cdot\text{H}^+]}$$

$$[\text{Bz}-\text{Py}\cdot\text{H}^+] = [\text{Bz}-\text{Py}] \times 10^{(\text{pKa1}-\text{pH})}$$

This equation shows that protonation of the pyridine moiety is strongly determined by the pK_a1 of PPI.

Amounts of [BzH^+−Py] at a given pH can be determined by measured pK_a2.

$$\text{PPI}\cdot 2\text{H}^+ \xrightarrow{K_a2} \text{PPI}\cdot\text{H}^+ + \text{H}^+$$

$$K_a2 = \frac{[\text{PPI}\cdot\text{H}^+][\text{H}^+]}{[\text{PPI}\cdot 2\text{H}^+]}$$

Since (Bz−Py)·H^+ is composed of two forms, Bz−Py·H^+ and Bz·H^+−Py, we may rewrite the equation as follows.

$$2(\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+) \rightarrow (\text{Bz}-\text{Py}\cdot\text{H}^+) + (\text{Bz}\cdot\text{H}^+-\text{Py}) + 2\text{H}^+$$

The protonation between Bz and Py is different, so we may separate the reaction using K_1 and K_2 as follows.

$$(\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+) \xrightarrow{K_1} (\text{Bz}\cdot\text{H}^+-\text{Py}) + \text{H}^+$$

$$(\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+) \xrightarrow{K_2} (\text{Bz}-\text{Py}\cdot\text{H}^+) + \text{H}^+$$

When the pH is near pK_a2, most of the pyridine moiety will be protonated, since the pK_a1 of the pyridine moiety of PPI is 3 pK_a units larger than pK_a2, which means that K_2 is similar to K_a2. So, we may simplify the calculation of [Bz·H^+−Py·H^+] in terms of pK_a2 instead of using pK_2 as follows.

$$(\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+) \xrightarrow{K_a2} (\text{Bz}-\text{Py}\cdot\text{H}^+) + \text{H}^+$$

$$K_a2 = \frac{[\text{Bz}-\text{Py}\cdot\text{H}^+][\text{H}^+]}{[\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+]}$$

$$[\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+] = [\text{Bz}-\text{Py}\cdot\text{H}^+] \times 10^{(\text{p}K_a2-\text{pH})} = [\text{Bz}-\text{Py}] \times 10^{(\text{p}K_a1+\text{p}K_a2-2\text{pH})}$$

When the Bz·H^+− part is ignored, the K_1 that is related to protonation of the pyridine moiety will be same as K_a1. The protonation of pyridine is expressed using pK_a1.

$$(\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+) \xrightarrow{K_a1} (\text{Bz}\cdot\text{H}^+-\text{Py}) + \text{H}^+$$

$$K_a1 = \frac{[\text{Bz}\cdot\text{H}^+-\text{Py}][\text{H}^+]}{[\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+]}$$

$$[\text{Bz}\cdot\text{H}^+-\text{Py}] = \frac{[\text{Bz}\cdot\text{H}^+-\text{Py}\cdot\text{H}^+]xK_a1}{[\text{H}^+]}$$

After [Bz·H^+−Py·H^+] was substituted with [Bz−Py],

$$[\text{Bz}\cdot\text{H}^+-\text{Py}] = [\text{Bz}-\text{Py}] \times 10^{(\text{pK}_a2-\text{pH})}$$

the amounts of BzH^+−Py or [BzH^+−Py] can be expressed approximately by pK_a2 at a given pH.

In the case of the imidazopyridine of tenatoprazole, the three nitrogens of tenatoprazole can be expressed as PyrIm−Py. The pyridine moiety of the pyridinylmethylsulfinyl group is the pK_a1, and the net protonation of the imidazo-pyridine is also the pK_a2′ of tenatoprazole. Again, this shows that the population of BzH^+−Py is more closely related to the pK_a2 (benzimidazole pK_a) or pK_a2′ (imidazole pK_a of tenatoprazole) than the pK_a1 (pyridine pK_a). These concepts will be illustrated by a comparison of lansoprazole and pantoprazole to illustrate the effect of different pK_a2's and then lansoprazole and rabeprazole to illustrate the effect of different pK_a1s.

Given that the pK_a of the pyridine ring is similar for lansoprazole (pK_a1 3.83) and pantoprazole (pK_a1 3.83), the relative amounts of free pyridine moiety of lansoprazole and pantoprazole will be similar at a given pH. Since the pK_a of the benzimidazole ring of lansoprazole (pK_a2 0.62) is higher than the pK_a2 of pantoprazole (pK_a 0.11) to the electron-withdrawing properties of the difluoromethoxy substituent, the concentration, [BzH^+−Py], of lansoprazole will be 3.24 ($10^{0.51}$) times higher than that of pantoprazole at a given pH. Since the unprotonated pyridine population of lansoprazole is similar to that of pantoprazole, we can expect a faster conversion of lansoprazole as compared to pantoprazole, since the proportion of the activated (protonated) benzimidazole moiety population of lansoprazole is higher than that of pantoprazole. Overall, the activation rate must be strongly affected by the second protonation related to the imidazole ring of benzimidazole pK_a2 of all the PPIs. When the second protonation, pK_a2, is similar, then differences of conversion rates of PPIs will be dependent on the relative nucleophilicity of pyridine and its pK_a1.

When the substituents of the pyridine moiety are different, the nucleophilicity of the unprotonated pyridine is different. The more nucleophilic pyridines will react faster than weaker nucleophilic pyridines. In the particular case of rabeprazole and lansoprazole, their benzimidazole moiety is identical; thus the pK_a of the second protonation on the benzimidazole ring and electrophilicity of C-2 of the benzimidazole ring will be very similar. So the difference of the conversion rate to active compounds must depend on differences in the pK_a1 of the pyridine moiety. The pyridine pK_a1 is 3.83 for lansoprazole and 4.53 for rabeprazole. Thus the free pyridine amounts will be higher in lansoprazole at a given pH as compared to rabeprazole. However, the nucleophilicity increase due to the methoxypropoxy substituent of rabeprazole will be greater than that of lansoprazole, since the trifluoroethoxy substituent of lansoprazole is a weaker electron-donating group compared to the methoxypropoxy group of rabeprazole. This increased nucleophilicity by the methoxypropoxy substitution will drive a faster conversion of rabeprazole at acidic pH thereby overcoming the smaller proportion of unprotonated pyridine. Under identical condition, when half-lives of lansoprazole and rabeprazole were compared at pH 5.1, the lansoprazole half-life was 1.5 h while that of rabeprazole was 0.12 h.[11] The conversion rate of rabeprazole was more than 10 times faster than that of lansoprazole due to the increased pyridine N nucleophilicity of this compound.

At pH 5.1, the unprotonated pyridine population of lansoprazole is about 94.8% and the unprotonated pyridine population of rabeprazole is about 78.8%. The protonated benzimidazole population of lansoprazole and rabeprazole will be below 0.01%

at their pK_a2 of 0.62. The difference of unprotonated pyridine population between lansoprazole and rabeprazole (about 16% higher in lansoprazole) is not significant when compared to the difference of nucleophilicity of the pyridine moiety between lansoprazole and rabeprazole. This shows that the nucleophilicity of pyridine is the main factor in determining the conversion rate when the population of activated benzimidazole by protonation is small.

In general, when the derivatives of the PPI structure sharing the same benzimidazole moiety but different substituents on the pyridine moiety were compared, the derivatives having higher pK_a1 showed a shorter half-life compared to the derivatives having lower pK_a1.[12,13,18] Since higher pK_a1 values suggest higher nucleophilicity together with higher basicity, the population of free pyridine will be decreased at a given pH, which will decrease the conversion rate, but increased nucleophilicity of the unprotonated fraction of the pyridine will increase the conversion rate, which overcomes the effect from the decreased population of unprotonated pyridine. The actual conversion rate will be determined by the nucleophilicity of free pyridine when the benzimidazole pK_a2 is the same for different PPIs. At pH 1.2, the lansoprazole half-life was 2 min while that of rabeprazole was 1.3 min.[11] At pH 1.2, the conversion rate seems to become equal. Compared to their half-lives at pH 5.1, conversion rates increased more than 40-fold for lansoprazole but only 5-fold for rabeprazole at pH 1.2. At pH 1.2, the protonated benzimidazole population of lansoprazole and rabeprazole was calculated to be about 21% with a pK_a2 of 0.62, which is now the dominant factor in determining the conversion rate. At pH 1.2, the free pyridine population is about 0.23% for lansoprazole and 0.05% for rabeprazole. Since about 21% of benzimidazole is now protonated at pH 1.2, 21% of the C-2 position of benzimidazole as an electrophile is ready to react with the free pyridine moiety that is nucleophilic. So, population of free pyridine at this pH (near pK_a2) is very important for the maximum conversion rate. Therefore, the lansoprazole conversion rate increases dramatically in acid in contrast to rabeprazole.

Conclusion

Generally, the conversion rates of the PPIs in acid are strongly affected by the second protonation at pK_a2 for the benzimidazoles (or the pK_a2′ for the imidazopyridine of tenatoprazole), which induces electrophilicity on the C-2 of benzimidazole and also by substituent effects on the nucleophilicity of pyridine moiety, pK_a1. However, the maximum conversion rates are mainly dependent on the second protonation of the benzimidazole ring that is reflected by the pK_a2. The effect of the second protonation determined by the pK_a2 on the conversion rate is very significant under acidic conditions (<pH 4) and the effect of pyridine nucleophilicity is significant at pH ≥ 4, since the C-2 position of the benzimidazole ring cannot behave as an electrophile at this pH. The pK_a1 of PPI shows why PPI is selectively accumulated in the parietal cell where the secretory canaliculus is the only membrane enclosed space with a pH < 4.0, and the pK_a2 of PPI shows why PPIs become activated on the proton pump in the canaliculus when acid secretion is stimulated to generate a highly acidic pH. Both factors combine to result in a highly specific reaction only with the active gastric (H^+,K^+)-ATPase in the body.

The binding of the protonated PPI occurs in to the vestibule of the pump close to cysteine 813. In the presence of proton transport by the pump, this protonated species is activated to the sulfenic acid. With fast activation, reaction occurs rapidly at cysteine 813 as has been shown for omeprazole, and further entrance into the membrane domain of the pump is prevented. With slow activation, such as found for pantoprazole or tenatoprazole, the protonated species can access cysteine 822 prior to activation and bind at both cysteines in the M5/M6 membrane domain.[7,9] This hypothesis would account for the known different cysteine reactivities of omeprazole and lansoprazole on one hand and for pantoprazole or tenatoprazole on the other.

Experimental Section

General. Chemicals, reagents, and solvents were purchased from Sigma-Aldrich (St. Louis, USA). 2-(Methylthio)benzimidazole purchased from Sigma Aldrich was crystallized from aqueous methanol. 5-Methoxy-*1H*-imidazo[4,5-*b*]pyridine-2-thiol was a gift from Negma-Lerads (Les Hameaux, France). NMR spectra were recorded on a 400 MHz instrument (ARX400) manufactured by Bruker. UV spectra were obtained by Shimadzu UV−visible spectrophotometer model UV-1601. The HPLC system was the Waters Breeze HPLC system (Waters, Milford, MA) with a UV detector, Waters 2487 Dual λ Absorbance detector.

Chromatography. Analytical HPLC was run on a Nucleosil C_{18} (250 mm × 4.6 mm, 5 μm) column using a mobile phase of 36% acetonitrile−20 mM sodium phosphate, pH 7.7, at a flow rate of 1 mL/min, and detection at 302 nm for omeprazole and N^1-methyl omeprazole. Other proton pump inhibitors were measured as follows. Lansoprazole and N^1-methyl lansoprazole were measured using a mobile phase of 38% acetonitrile−20 mM sodium phosphate, pH 7.7, at a flow rate of 1 mL/min, and detection at 284 nm for lansoprazole and 285 nm for N^1-methyl lansoprazole. Pantoprazole, rabeprazole, tenatoprazole, N^1-methyl tenatoprazole, and N^1-pantoprazole were measured at 291, 284, 311, 311, and 289 nm, respectively, using a mobile phase 35% acetonitrile in 20 mM sodium phosphate, pH 7.7.

Synthesis of 2-Methylsulfinyl-1*H*-benzimidazoles. A. General Method of Synthesis. To a solution of 2-methylthio-1*H*-benzimidazole (20 mmol) in 100 mL of dichloromethane and 10 mL of methanol at −30 °C was added mCPBA (6 g in 100 mL of dichloromethane, 60% purity). The reaction mixture was stirred at −30 °C for 1 h and then twice washed with 200 mL of saturated $NaHCO_3$. The methylene chloride layer was separated, washed with saturated NaCl solution, and dried over anhydrous $MgSO_4$. CH_2Cl_2 was evaporated under reduced pressure. The residue was crystallized from benzene−hexane to give 2.7 g of 2-methylsulfinyl-*1H*-benzimidazole (75% yield).

B. 2-Methylsulfinyl-1*H*-benzimidazole (3a) (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.21 (s, 3 H), 7.33 (m, 2 H), 7.70 (m, 2 H). HRMS (EI): calculated for $C_8H_8N_2OS$ [M^+] *m*/*z* 180.0357 was found to be 180.0350.

C. 5-Methoxy-2-methylsulfinyl-1*H*-benzimidazole (3b) (73.7% Yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (s, 3 H), 3.84 (s, 3 H), 6.96 (d, 1 H), 7.08 (s, 1 H), 7.58 (d, 1 H). HRMS (EI): calcd for $C_9H_{10}N_2O_2S$ [M^+] *m*/*z* 210.0463, found 210.0466.

C. 5-Difluoromethoxy-2-methylsulfinyl-1*H*-benzimidazole (3c) (75.1% Yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.21 (s, 3 H), 6.54 (t, J = 74.0 Hz, 1 H), 7.14 (d, 1 H), 7.45 (s, 1 H), 7.65 (d, 1 H). HRMS (EI): calcd for $C_9H_8F_2N_2O_2S$ [M^+] *m*/*z* 246.0275, found 246.0279.

D. 2-Methanesulfinyl-5-methoxy-1*H*-imidazo[4,5-*b*]pyridine (3d) (80.5% Yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (s, 3 H), 3.99 (s, 3 H), 6.76 (d, 1 H), 7.88 (d, 1 H). HRMS (EI): calcd for $C_8H_9N_3O_2S$ [M^+] *m*/*z* 211.0415, found 211.0414.

Synthesis of 1-Methyl-2-methylsulfinylbenzimidazoles (4). A. General Method of Synthesis. To a solution of 2-methylsulfinyl-1*H*-benzimidazole and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene, 1.2 equiv) in CH_2Cl_2 was added a solution of dimethyl sulfate (2.0 equiv) in CH_2-

Cl_2 at room temperature, and then the mixture was stirred for 30−60 min until completion of the reaction. After water was added, the mixture was stirred vigorously for 30 min. Then the mixture was purified, without further treatment, by short silica gel column chromatography (CH_2Cl_2 to 2% MeOH in CH_2Cl_2) to give 1-methyl-2-methylsulfinylbenzimidazole as colorless oil.

N-Methylation of 5-methoxy-2-(methylsulfinyl)benzimidazole and 5-difluoromethoxy-2-(methylsulfinyl)benzimidazole gave an inseparable mixture of 5- and 6-methoxy isomers of 1-methyl 2-(methylsulfinyl)-benzimidazole and a mixture of 5- and 6-difluoromethoxy isomers of 1-methyl 2-(methylsulfinyl)benzimidazole, respectively.

B. 1-Methyl-2-methylsulfinylbenzimidazole (4a), Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.26 (s, 3 H), 4.13 (s, 3 H), 7.35−7.45 (m, 2 H), 7.82 (d, 1 H). HRMS (EI): calculated for $C_9H_{10}N_2OS$ [M^+] *m*/*z* 194.0514, found 194.0512.

C. 5- and 6-Methoxy-1-methyl-2-methylsulfinylbenzimidazole (4b) (a Mixture of 5-MeO- and 6-MeO-derivatives; Ratio ≈ 1:1), Yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.22 and 3.23 (s, 3 H), 3.85 and 3.88 (s, 3 H), 4.06 and 4.08 (s, 3 H), 6.56 (m), 6.79 (m), 7.00 (dd), 7.25 (m), 7.66 (d). HRMS (EI): calculated for $C_{10}H_{12}N_2O_2S$ [M^+] *m*/*z* 224.0620, found 224.0622.

D. 5- and 6-Difluoromethoxy-1-methyl-2-methylsulfinylbenzimidazole (4c) (a Mixture of 5-CHF_2O- and 6-CHF_2O-derivatives; Ratio ≈ 1:1), Yield 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.26 (s, 3 H), 4.12 and 4.14 (s, 3 H; N-Me), 6.54 and 6.56 (t, J = 74.0 Hz, 1 H), 7.15−7.26 (m), 7.41 (d), 7.58 (s), 7.79 (d). HRMS (EI): calculated for $C_{10}H_{10}F_2N_2O_2SNa$ [M + Na^+] *m*/*z* 283.0320, found 283.0323.

Synthesis of *N*^{1}-Methyl Proton Pump Inhibitors (6). A. General Method of Synthesis. To a solution of PPI and DBU (1,8-diazabicyclo-[5.4.0]undec-7-ene, 1.2 equiv) in CH_2Cl_2 was added a solution of dimethyl sulfate (2.0 equiv) in CH_2Cl_2 at room temperature, and then the mixture was stirred with monitoring the reaction by thin-layer chromatography. After the reaction finished, water was added to the reaction mixture, which was stirred vigorously for 30 min. Then the mixture was extracted with CH_2Cl_2, and the organic layer was washed with 1 N $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, and concentrated on a rotary evaporator. The resulting oil was purified by short silica gel column chromatography (CH_2Cl_2 to 2% MeOH in CH_2Cl_2) to give *N*^{1}-methyl PPI as a white solid.

B. *N*^{1}-Methyl Omeprazole (6a) (a Mixture of 5-MeO- and 6-MeO-derivatives; Ratio ≈ 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (s, 3 H), 2.28 (s, 3 H), 3.69 and 3.70 (s, 3 H), 3.85 and 3.86 (s, 3 H), 3.93 and 3.94 (s, 3 H), 4.93 (s, 2 H), 6.77 (s), 6.95 (dd), 7.02 (dd), 7.25 (m), 7.66 (d), 8.11 (s). HRMS (FAB): calculated for $C_{18}H_{22}N_3O_3S$ [MH^+] *m*/*z* 360.1382, found 360.1391.

C. *N*^{1}-Methyl Lansoprazole (6b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31 (s, 3 H), 4.03 (s, 3 H), 4.37 (q, 2 H), 5.00 (dd, 2 H), 6.64 (d, 1 H), 7.35 (m, 3 H), 7.80 (d, 1 H), 8.23 (d, 1 H). HRMS (FAB): calculated for $C_{17}H_{17}F_3N_3O_2S$ [MH^+] *m*/*z* 384.0994, found 384.1008.

D. *N*^{1}-Methyl Rabeprazole (6c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (q, 2 H), 2.26 (s, 3 H), 3.34 (s, 3 H), 3.53 (t, 2 H), 4.02 (s, 3 H), 4.09 (t, 2 H), 4.96 (s, 2 H), 6.70 (d, 1 H), 7.33 (m, 1 H), 7.38 (m, 1 H), 7.82 (d, 1 H), 8.17 (d, 1 H). HRMS (FAB): calculated for $C_{19}H_{24}N_3O_3S$ [MH^+] *m*/*z* 374.1538, found 374.1531.

E. *N*^{1}-Methyl Pantoprazole (6d) (a Mixture of 5-CHF_2O- and 6-CHF_2O-derivatives; Ratio ≈ 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 3 H), 3.91 (s, 3 H), 4.01 and 4.04 (s, 3 H), 4.88−5.00 (m, 2 H), 6.52 and 6.56 (t, J = 74.0 Hz, 1 H), 6.79 (d), 7.20 (m), 7.38 (d), 7.55 (s), 7.76 (d), 8.09 (m). HRMS (FAB): calculated for $C_{17}H_{18}F_2N_3O_4S$ [MH^+] *m*/*z* 398.0986, found 398.0974.

F. *N*^{1}-Methyl Tenatoprazole (6e) (a Mixture of 5-MeO- and 6-MeO-derivatives, Ratio ≈ 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (s, 3 H), 2.21 (s, 3 H), 2.27 (s, 3 H), 2.31 (s, 3 H), 3.70 (s, 3 H), 3.74 (s, 3 H), 3.96 (s, 3 H), 3.99 (s, 3 H), 4.02 (s, 3 H), 4.05 (s, 3 H), 4.91 (dd, 2 H), 5.02 (dd, 2 H), 6.71 (d, 1 H), 6.80 (d, 1 H), 7.64 (d, 1 H), 7.91 (d, 1 H), 8.10 (m, 1 H), 8.11 (m, 1 H); HRMS (FAB): calculated for $C_{17}H_{20}N_4O_3SNa$ [M + Na^+] *m*/*z* 383.1151, found 383.1148.

Determination of pK_a. A. UV Spectroscopy Method. The compounds at a concentration of 0.1−0.12 mM were dissolved in a buffer composed of 10 mM Tris/HCl buffer (pH 8 to pH 6), 10 mM sodium citrate buffer (pH 2.53 to pH 5.6), and HCl solution (5 mM to 5 M) and scanned at room temperature (25 °C) within 25 s using the Shimadzu UV-1601 UV−visible spectrophotometer.

B. Titration Method. The compounds (5 to 10 mM) in 5−10% aqueous methanol solution were titrated with HCl.

C. Calculation of pK_a from Spectral Data. Generally, the pK_a of PPI can be calculated as follows.

$$\mathrm{PPI{\cdot}H^+} \xrightarrow{K_a1} \mathrm{PPI} + \mathrm{H^+}$$

$$K_a1 = \frac{[\mathrm{PPI}][\mathrm{H^+}]}{[\mathrm{PPI{\cdot}H^+}]} = [\mathrm{H^+}] \times \frac{(di - d)}{(d - dm)}$$

So,

$$\mathrm{p}K_a1 = \mathrm{pH} + \log\left(\frac{dm - d}{d - di}\right)$$

where *dm* is pure nonionic absorbance (Abs) at a given analytical wavelength and *di* is pure monocationic absorbance at a given analytical wavelength.

$$\mathrm{PPI{\cdot}2H^+} \xrightarrow{K_a2} \mathrm{PPI{\cdot}H^+} + \mathrm{H^+}$$

$$K_a2 = \frac{[\mathrm{PPI{\cdot}H^+}][\mathrm{H^+}]}{[\mathrm{PPI{\cdot}2H^+}]}$$

$$K_a2 = [\mathrm{H^+}] \times \frac{(d - di)}{(dii - d)}$$

So,

$$\mathrm{p}K_a2 = \mathrm{pH} + \log\left(\frac{d - di}{dii - d}\right)$$

where *di* is the pure monocationic absorbance and *dii* is the pure dicationic absorbance at given analytical wavelength.

In this experiment, *dm* is easily measured where Abs is of nonionic species (in this example, Abs was mostly obtained at pH 5.6).

The absorbance at an analytical wavelength, *d*, can be rewritten as follows.

$$\mathrm{d(observed)} = \text{Abs by amounts of di} + \text{Abs by amounts of dii}$$

If there is a big difference between pK_a1 and pK_a2, *di* and *dii* will be easily measured by observed Abs, *d*, at a given pH.

In this experiment, the absolute values of di and dii are not known; however we can measure absorbance near *di* and *dii*, since the approximate pK_a1 and pK_a2 showed greater than a 3 pK_a unit difference, which suggests that observed *di* or *dii* would contain less than 0.1% of mono- or di-ionic species contamination at a given pH near pK_a1 or pK_a2.

However, we calculate *di* from measured Abs, *d*. For example, *di* can be calculated by two absorbances, *d*1*a* and *d*1*b*, measured at a given pH*a* and pH*b* at the analytical wavelength, *D*1 nm.

$$K_a1 = \frac{[\mathrm{PPI}][\mathrm{H^+}]}{[\mathrm{PPI{\cdot}H^+}]} = [\mathrm{H^+}] \times \frac{(di - d)}{(d - dm)}$$

Therefore,

$$K_a1 = [\mathrm{H^+}]a \times \frac{(di - d1a)}{(d1a - dm)} = [\mathrm{H^+}]b \times \frac{(di - d1b)}{(d1b - dm)}$$

where *dm* is pure nonionic Abs and *di* is pure monocationic Abs at a given wavelength, *D*1, and *d*1*a* is measured Abs at given pH*a* and d1b is measured Abs at a given pH*b*

$$\mathrm{p}K_a1 = \mathrm{pHa} + \log\left(\frac{dm - d1a}{d1a - di}\right) = \mathrm{pHb} + \log\left(\frac{dm - d1b}{d1b - di}\right)$$

$$\mathrm{p}K_a1\text{-pHa} = \log\left(\frac{dm - d1a}{d1a - di}\right) \quad (1)$$

$$\mathrm{p}K_a1\text{-pHb} = \log\left(\frac{dm - d1b}{d1b - di}\right) \quad (2)$$

$$\mathrm{pHb\text{-}pHa} = \log\left(\frac{dm - d1a}{d1a - di}\right)\text{-}\log\left(\frac{dm - d1b}{d1b - di}\right) \quad (3)$$

Let $a = \mathrm{pH}b - \mathrm{pH}a$, $b = dm - d1a$, $c = dm - d1b$, $k = 10^{(\mathrm{pH}b - \mathrm{pH}a)} = 10^a$,

$$k = \left(\frac{dm - d1a}{d1a - di}\right) \times \left(\frac{d1b - di}{dm - d1b}\right) = \left(\frac{b}{d1a - di}\right) \times \left(\frac{d1b - di}{c}\right) \quad (4)$$

$$di = \frac{\frac{kc}{b}\cdot d1a - d1b}{\frac{kc}{b} - 1} \quad (5)$$

Now, *di* is determined by observed Abs at pH*a* and pH*b* and pK_a1 can be calculated.

When *d*1*a* and *d*1*b* are measured at pH*a* and pH*b* where pHa and pHb are far from pK_a2, observed Abs, optical density, *d*1*a* and *d*1*b* show only the absorbance of the amounts of monocationic species at given pH*a* and pH*b*.

Instead of obtaining pure dicationic absorbance, *dii*, at strong acidity, we can determine pK_a2 by measuring two different absorbances, *d*2*a* and *d*2*b*, at pHa and pHb as follows.

$$K_a2 = [\mathrm{H}^+] \times \frac{(dii - d2)}{(d2 - di)}$$

where *di* is pure monocationic Abs. K_a2 can be calculated as follows.

Optical density, *d*2*a* was observed at pH*a* and *d*2*b* was observed at pH*b*.

$$K_a2 = [\mathrm{H}^+]a \times \frac{(dii - d2a)}{(d2a - di)} = [\mathrm{H}^+]b \times \frac{(dii - d2b)}{(d2b - di)} \quad (6)$$

$$dii - d2a = \frac{(d2a - di)xK_a2}{[H^+]a}$$

$$dii - d2b = \frac{(d2b - di)xK_a2}{[H^+]b}$$

After eliminating *dii*, eq 6 can be rearranged as follows.

$$\frac{(d2a - di)xK_a2}{[H^+]a} - \frac{(d2b - di)xK_a2}{[H^+]b} = d2b - d2a \quad (7)$$

$$K_a2 = \frac{(d2b - d2a)}{(d2a - di)/[H^+]a - (d2b - di)/[H^+]b} \quad (8)$$

$$\mathrm{p}K_a2 = -\log(K_a2)$$

At given pH*a* and pH*b*, absorbance *d*2*a* and *d*2*b* provide the K_a2.

Stability of Proton Pump Inhibitors. PPI stability was measured at various pH values in the absence of thiol compounds as follows. The compounds were dissolved in methanol at a concentration of 20−50 mM as stock solution. An aliquot was taken from the stock solution and added to a buffer solution (pH 1.3 to 8.0) with a final concentration of the compounds of 0.1−0.25 mM. The final methanol concentration was 0.5%.

The buffer solutions between pH 1.3 and 8.0 were made as follows: pH 1.3, 20 mM KCl by HCl adding up to pH 1.3; pH 2.3, 20 mM Glycine; pH 3.1; 10 mM citric acid, 5 mM Na_2HPO_4; pH 4.1, 10 mM citric acid, 12 mM Na_2HPO_4; pH 5.1, 10 mM citric acid, 20 mM Na_2HPO_4; pH 6.1, 10 mM citric acid, 35 mM Na_2HPO_4; pH 7.1, 10 mM citric acid, 90 mM Na_2HPO_4; pH 7.4, 10 mM citric acid, 180 mM Na_2HPO_4; pH 8.0, 100 mM sodium phosphate buffer.

The buffer solution containing 0.1−0.25 mM of the compounds was incubated at 25 °C in the absence of thiol compounds. At timed intervals, an aliquot (100 μL) was taken and added to 2.5 mL of 0.2 M Na_2HPO_4 buffer, pH 8.8, to stop the conversion. An aliquot was taken and analyzed by HPLC.

The conversion rate under acidic pH was measured using HCl solutions: 20 mM, 50 mM, 0.1 M, 0.2 M, 0.3 M, 0.5 M, 1.0 M, 2.0 M, 4.0 M, and 5 M.

The compound was made at a concentration of 0.5 mM in a given HCl solution, and an aliquot (50 μL) was taken and added to 5 mL of 0.2 M Na_2HPO_4 buffer, pH 8.8, and immediately analyzed by HPLC.

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Note Added after ASAP Posting

After this paper was posted ASAP on 06/04/2004, Figures 2 and 4, and their respective captions, were corrected to indicate that the y-axis in each figure shows the conversion rate constant. The corrected version was posted 06/10/2004.

Supporting Information Available: Experimental details of synthesis and UV-spectra of PPIs. This material is available free of charge via the Internet at http://pubs.acs.org.

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