

RESEARCH ARTICLE

Mechanochemical synthesis and *in vitro* anti-*Helicobacter pylori* and urease inhibitory activities of novel zinc(II)–famotidine complex

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Abstract

The mechanochemical synthesis and characterization of a zinc complex with famotidine is described. The complex was characterized by microanalysis and a number of spectroscopic techniques. The complex was of M:L dihydrate type. Derivatization of famotidine with zinc appears to enhance the activity of the drug by inhibiting the growth of *Helicobacter pylori* (two reference and 34 clinical isolates). The complex inhibited the growth of *H. pylori* in an MIC range of 1–8 $\mu\text{g mL}^{-1}$. The anti-*H. pylori* activity of the zinc–famotidine complex against antibiotic-resistant strains was nearly comparable to that of antibiotic-susceptible strains. The complex was found to be far less toxic than the parent drug, as demonstrated by its higher LD₅₀ value. In the human urease enzyme inhibition assay the complex exhibited significant inhibition. The new complex appears to be more useful in eradicating both the antibiotic-susceptible and antibiotic-resistant strains of *H. pylori*.

Keywords: Zinc complexes; zinc–famotidine; antiulcer drugs; metal-based drugs; anti-*H. pylori* activity; mechanochemical synthesis; urease inhibition

Introduction

Famotidine, 3-([2-diaminomethyleneamino)thiazol-4-yl]-methylsulfanyl-*N*-sulfamoyl-propionamide), is an effective antiulcer drug having an excellent histamine H₂ receptor blocking effect. Like others, this drug is not free from side effects. Medicinal chemists are making continued efforts to enhance the activity of these drugs through preparation of their suitable derivatives. In this context famotidine has been reported to be complexed with copper¹, nickel², and cobalt³. These metals are known to possess high toxicity. Zinc is among biologically friendly trace elements, and several zinc-based drugs are in use—the most prominent being polaprezinc, a very effective antiulcer drug⁴. Zinc has a well-established role in wound healing⁵. On account of this property and its relatively lower toxicity, zinc has found

a place in the design of metal-based drugs. Moreover it has been demonstrated that chelation of a drug molecule with a metal ion can enhance its efficacy⁶. Keeping in view these properties, zinc has been complexed with several drug substances, including antiulcer drugs such as cimetidine and ranitidine^{7,8}. The complexation is usually performed in solution by use of organic solvents, which may end up as residual solvents in the final product. The presence of residual organic solvents in the drug substances is highly undesirable due to their high toxicity and, as such, pharmacopeias and the International Conference on Harmonisation⁹ place a limit on this. It is therefore desirable that the drug substances be made solvent free. Recently, methods have been developed for solvent-free synthesis of metal complexes¹⁰ which have promise in pharmaceutical manufacture. In this study we

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report the preparation of the title compound by use of a solvent-free mechanochemical method.

It is now firmly established that gastric and duodenal ulcers are generally caused by *Helicobacter pylori*, which survives and grows in acidic environments¹¹. Triple therapy, including a proton pump inhibitor and any of two antibiotics such as amoxicillin (AMX), clarithromycin (CLT), metronidazole (MNZ), and tetracycline (TET), is frequently conducted to eradicate *H. pylori*. Many clinical trials have reported an eradication rate of about 80–90% by using a relevant triple therapy¹². However, many concerns, including the emergence of antibiotic-resistant strains of *H. pylori* due to overuse and adverse effects of antibiotics, are yet to be addressed. Therefore, there is a need to develop antimicrobial agents with enhanced efficacy and reduced toxicity.

In the present article we report the solvent-free synthesis of a zinc(II)–famotidine complex using a mechanochemical method and its *in vitro* anti-*H. pylori* and urease inhibitory activity.

Materials and methods

Materials

All chemical reagents were of analytical grade (Sigma-Aldrich, UK) and solvents were of extra pure grade (Fischer Scientific, UK), and used without further purification. Human urease was obtained from Gesellschaft für Biochemica und Diagnostica GmbH, Germany. Deuterated dimethyl sulfoxide (DMSO- d_6) was obtained from Cambridge Isotope Laboratories, Inc., USA.

Synthesis

The appropriate drug (1 mmol) and zinc acetate dihydrate (0.5 mmol) were ground using an agate pestle and mortar for 30–40 s; a microcrystalline powder of white color was obtained. The product was characterized both without further washing and after washing with methanol.

Characterization

Electronic absorption spectra of the metal complex were recorded as diffuse reflectance on a Lambda 35 UV/Vis spectrophotometer in the 200–900 nm range. The background spectrum of the glass plate employed was subtracted using the instrument's software. Infrared (IR) spectra of the solid compounds were recorded by the reflectance method on a PerkinElmer Spectrum 100 FT-IR system. Proton and ¹³C nuclear magnetic resonance (NMR) spectra were recorded in DMSO- d_6 on a Jeol machine operating at 270 MHz and a Delta machine operating at 400 MHz, respectively, at 298 K. NMR tubes with a 5-mm internal diameter were used for all experiments. Tetramethylsilane (TMS) and the residual protonated solvent peak (DMSO- d_6 at 2.5 ppm) were used to calibrate the chemical shift.

Powder X-ray diffraction (PXRD) spectra of the compound were recorded on a Bruker D8 Avance machine, with CuK α radiation. The PXRD spectra of the free ligand (pure drug) were compared with those of the complex. C, H, and

N analyses were carried out using a Eurovector EA 3000 elemental analyzer. The melting/decomposition point of the complex was determined using a Gallenkamp melting point apparatus. Thermal analysis was carried out by the use of a TGA Q 500 V6.7 build 203 instrument in thermogravimetry (TGA) mode from ambient to 500°C. Solubility of the complex was determined in hot and cold water, *N,N*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and other common organic solvents by shaking a small amount of compound in the solvent in a test tube. The mass spectrum of the complex was recorded on a Q Star mass spectrometer using nano-electrospray ionization (ESI)-expansion technique.

Isolation of *H. pylori*

Reagents

Brain heart infusion broth (CM 225), Columbia agar (CM 331), Müller–Hinton agar base, blood agar base (CM 55), Brucella agar, fetal bovine serum, Campylobacter selective supplement Skirrow, SR 69 consisting of vancomycin (5 mg), polymyxin (1250 IU), and trimethoprim (2.5 mg), micro-aerobic atmosphere (5% O₂, 10% CO₂, and 85% N₂) created by a Campygen sachet (CN 25), and gas jar anaerogen (AN 25) were purchased from Oxoid, UK. Methanol, ethanol, and diethyl ether were from Fischer Scientific, UK.

H. pylori strains

A total of 34 local strains of *H. pylori* were isolated from biopsies obtained from Allied Hospital, a teaching hospital of Punjab Medical College, Faisalabad, using standard protocols. *H. pylori* reference strains NCTC 11637 and NCTC 11638 were obtained from the National Health Protection Agency, London, UK.

Procedure

Patients

Patients who reported to the gastroendoscopy unit of Allied Hospital, Faisalabad and associated clinics for upper gastro-duodenal endoscopy during 2008–2009 were included in this study. Patient history was taken as per standard practice. Patients who had been using non-steroidal anti-inflammatory drugs (NSAIDs) and any antibiotics 3 months before the study were excluded. The patients (51) admitted to this study were 18–89 (median 34.5) years of age. Biopsy samples were taken from different parts of the upper gastrointestinal tract with emphasis on biopsies from the duodenum and antrum.

Culture of biopsy samples

Gastric biopsy samples were kept in sterile tubes containing transport medium, consisting of brain heart infusion broth with 5% fetal bovine serum supplemented with Campylobacter selective supplement. The biopsies were transported on the same day with dry ice.

All manipulations were performed in a laminar flow cabinet. The biopsy tissue was placed on the frosted end of a sterilized microscope slide. Approximately 100 μ L of brain heart infusion broth was added to the biopsy tissue on the slide

and the tissue was ground between two slides to homogenize. The ground samples were divided into two halves, one half for inoculation on media plates and the other half for histological studies and urease test. The homogenized tissue from each biopsy was inoculated onto Columbia agar with 5% fetal bovine serum supplemented with Campylobacter selective supplement. The plates were incubated at 37°C under microaerobic conditions for 72 or 96 h as appropriate. Then the plates were observed for growth. The plates that did not show growth were reincubated. A part of the other half of the homogenized biopsy sample was observed under an optical microscope for the presence of *H. pylori*, while the remaining part was used for urease test and Gram's staining.

Characterization of the cultures

The isolates were also subjected to urease, catalase, and oxidase tests according to Cruickshank *et al.*¹³. A specimen was considered to be *H. pylori* positive if it was identifiable by Gram's staining, Giemsa's staining, urease, catalase, and oxidase positive, and morphological tests. The analytical profile index (API) of *H. pylori* was determined using API Campy (BioMerieux, France) according to its instruction manual. *H. pylori* motility testing was carried out according to the general methods described for motile bacteria¹³. Stock cultures were stored in brain heart infusion broth supplemented with 15% glycerol at -85°C in a freezer.

Anti-*H. pylori* activity (in vitro)

The agar dilution method according to the guidelines provided by the US National Committee for Clinical Laboratory Standards was used for antimicrobial tests. The frozen clinical isolates were thawed and diluted using Müller-Hinton infusion broth and adjusted to 10⁷ cfu mL⁻¹. A standardized loop (dia: 1 mm; streak: 2 cm) was used to seed the bacterial suspension onto the plates. Fourteen wells on a 96-well plate were filled with two-fold serially diluted test compound having final concentrations of 1024–0.125 µg mL⁻¹ in DMSO. The control well was filled with DMSO only. These dilutions were transferred to the media and inoculated with test culture, and inverted plates were incubated under microaerophilic conditions at 37°C for 72 or 96 h as appropriate. Minimum inhibitory concentrations (MICs) were then determined as per standard procedure. The breakpoints to define a resistant strain in this study, according to Megraud *et al.*¹⁴, were: metronidazole 8 µg mL⁻¹, clarithromycin 1 µg mL⁻¹; and according to Wu *et al.*¹⁵: 0.5 µg mL⁻¹ and 16 µg mL⁻¹ for amoxicillin and tetracycline, respectively.

Urease inhibitory activity (in vitro)

Several authors report urease activity under the influence of various medicinal compounds by use of slightly varying methods^{160–28}. In the present work the urease inhibitory activity of the complex was determined by a modified Berthelot (phenolphosphorite) method²⁹. One unit of human urease (Gesellschaft für Biochemica und Diagnostica GmbH, Germany) in 200 mL of reagent 1 (120 mmol phosphate

buffer pH 7.0, 60 mmol sodium salicylate, 5 mmol sodium nitroprusside, and 1 mmol ethylenediaminetetraacetic acid (EDTA) per L) was mixed with 600 mL of phosphate buffer and activated at 258°C for 10 min. This was followed by the addition of 20 µL of the test solution containing 1–8 µM of test compound in DMSO. DMSO (20 µL) was used as a control, and it was found that it did not show any inhibitory effect on the activity of the enzyme. The mixture was allowed to stand for 10 min to allow for interaction of the test compound with the enzyme. In order to achieve a final concentration of 1.5 mM urea per reaction, 150 mL of 20 mM urea in phosphate buffer (pH 7.0) was added to each reaction mixture except the calibration mixture, where the same volume of the phosphate buffer alone was added. The urea-blank mixture was used to normalize against optical density contribution by the test compound itself. The reaction mixture was incubated for an additional 10 min at 258°C to accomplish urea hydrolysis. The reaction was stopped by adding 1 mL of reagent 2 (120 mmol phosphate buffer pH 13 and 0.6 g hypochlorite per L). The ammonia liberated was allowed to complex with the hypochlorite and salicylate for 25 min and estimated by recording absorbance at 578 nm. Results were compared with thiourea, a standard urease inhibitor. The percentage inhibition was calculated as the difference between absorbance values with and without the test compounds³⁰.

Toxicity study

The LD₅₀ value of the complex under investigation was determined as follows by a reported method³¹ and compared with the literature value of the parent drug³². The experiments were conducted according to the protocol approved by the committee of the laboratory animals safety and public health ethical concerns of the University of Agriculture, Faisalabad, Pakistan. Male albino Wistar rats (average weight 288 ± 3.2 g; age 72–112 days) were used. During the experiments the animals were kept in metal cages at 23 ± 2°C and 55 ± 2% humidity. The animals were randomly divided into 10 dose groups (10 animals each). The drugs were administered as a suspension (5 mL) in edible oil orally with the help of disposable syringes, taking care to avoid dripping the drug suspension into the trachea by holding the mouth firmly with the hands. The animals were allowed to have laboratory feed and water *ad libitum*. The animals were monitored regularly and their body temperature was recorded on daily basis for 7 days after drug administration. A mortality and health record was maintained for each group.

Results and discussion

Synthesis of the complex

The zinc-famotidine complex was synthesized by a solvent-free mechanochemical technique. A white crystalline powder was isolated as such and after washing with methanol.

On grinding the drug with zinc acetate dihydrate, during preparation by the solvent-free method, acetic acid was

released, which was identified by the vinegar-like smell. Completion of reaction was ascertained by cessation of acetic acid fumes. The time required for completion of the reaction was found to be 2–3 min. The process using zinc chloride instead of zinc acetate produced the same results with the evolution of hydrochloric acid fumes, which were identified by bringing an ammonia-dipped rod close to the pestle and mortar.

Microanalytical data along with physical properties are listed in Table 1. The product thus obtained was characterized by Fourier-transform infrared (FT-IR) and electronic spectroscopy, nuclear magnetic resonance (^1H NMR and ^{13}C NMR), PXRD, mass spectrometry, and TGA. The C, H, N, and Zn analyses (Table 1) agreed with the proposed composition $\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$.

IR spectra and mode of bonding

Bonding of the drug (ligand) to zinc was investigated by comparing FT-IR spectroscopy of the complex with that of the free ligand. The spectrum of the complex contained all the absorption bands due to the ligand molecule, and some new absorption bands indicative of coordination of the ligand with the zinc ion also appeared. The important characteristic bands along with assignments are listed in Table 2. The famotidine molecule has a number of functional groups which can take part in coordination. A comparison of the FT-IR spectra of the ligand and the complex indicate coordination through guanidine- NH_2 , sulfamoyl- NH_2 , $\text{NH}_2\text{-C}=\text{N}$, and thiazole ring-N. The guanidine- NH_2 and sulfamoyl- NH_2 groups appear to deprotonate also. These changes are associated with the disappearance and/or shifting of relevant absorption frequencies as shown in Table 2.

UV-visible spectrum

The electronic absorption spectrum of the complex in the ultraviolet (UV)-visible region contained the charge-transfer band at 325 nm and a band at 275 nm ($\pi\text{-}\pi^*$ transition) due to the chromophore in the ligand molecules, which indicates the presence of the drug moiety in the complex.

Table 1. Physical and microanalytical data of $\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$ ($\text{C}_8\text{H}_{13}\text{N}_7\text{O}_2\text{S}_3\text{Zn}\cdot 2\text{H}_2\text{O}$).

	Color	Decomposition/melting point ($^\circ\text{C}$)	% Found (calculated)			
			C	H	N	Zn
Without washing	White	>246	21.77(21.99)	3.82(3.92)	22.25(22.44)	14.65(14.97)
After washing	White	>246	21.82(21.99)	3.72(3.92)	22.28(22.44)	14.85(14.97)

Table 2. Some of the characteristic observed infrared frequencies (cm^{-1}) and assignments.

Compound	$\nu(\text{NH}_2)_{\text{gua}}$	$\nu(\text{NH}_2)_{\text{sulfa}}$	$\nu(\text{N-C}=\text{N})$	$\nu(\text{thiazole ring})$
Famotidine	3505, 3474	3348, 3398, 3233	1530	1488, 1427, 1409
$\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$ (without washing)	3360	3255	1521	1403
$\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$ (after washing)	3361	3255	1523	1401

^1H NMR and ^{13}C NMR spectra

The ^1H NMR spectrum of the complex is detailed in Table 3. The chemical shifts are reported as δ (ppm) downfield from TMS. The numbering scheme is as shown in Figure 1. In the complex, the $\text{SO}_2\text{-NH}_2$ and $\text{N}=\text{C}(\text{NH}_2)_2$ groups become deprotonated and bonded to Zn as evidenced by the disappearance and weakening of their signals at about 8.2 and 6.8 ppm, respectively, in the spectrum of the free drug. All other proton signals in the spectrum of the free drug were also present in the spectrum of the complex, with slight variation in their chemical shifts (Table 3). The ^{13}C -NMR spectrum of the complex is detailed in Table 3. The chemical shifts are reported as δ (ppm) downfield from TMS. The spectrum of the complex contained the same number of peaks as in the spectrum of the free drug, confirming the presence of the full ligand skeleton in the complex. The spectrum supports the proposed composition and structure of the complex.

Powder X-ray diffraction

The PXRD patterns of the zinc complex, with and without washing, were found to be identical and significantly different from that of the parent drug, the free ligand.

TGA of the complex

Thermal decomposition of the complex was studied, under a nitrogen atmosphere, using TGA techniques. There was a weight loss (8.24%) at 120–200 $^\circ\text{C}$, which was equivalent to the loss of two water molecules. This weight loss indicates the presence of coordinated water in the complex, as the lattice water is usually lost at around 100 $^\circ\text{C}$. There was another weight loss (76.78%) at 200–500 $^\circ\text{C}$ corresponding to the loss of ligand.

Mass spectra of the complex

The molecular ions along with various fragments appearing in the mass spectrum are shown in Figure 2. The presence of the molecular ion peak and peaks representing the fragments clearly supports the proposed composition of the complex. As the spectrum was obtained by use of DMSO as the solvent, the fragments appearing in the spectrum

Table 3. NMR data (δ , ppm) of the complex.

Complex	^1H	^{13}C
$\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$ (without washing)	6.48–6.47 (d, aromatic), 3.59 (s, H9) 2.67 (s, H8), 2.67–2.64 (m, H6), 2.48–2.44 (m)	C2 (138), C4 (140), C5 (130), C7 (135), C9 (18), C11 (56), C12 (18), C8 (54)
$\text{Zn}(\text{famotidine})\cdot 2\text{H}_2\text{O}$ (after washing)	6.48–6.47 (d, aromatic), 3.56 (s, H9) 2.67 (s, H8), 2.67–2.61 (m, H6), 2.49–2.44 (m)	C2 (138), C4 (140), C5 (130), C7 (135), C9 (18), C11 (56), C12 (18), C8 (54)

indicate the replacement of water molecules with DMSO. Based on these results, the proposed structure of the complex is shown in Figure 3.

Isolation of *H. pylori*

The *H. pylori* cultures were identified as Gram-negative, and oxidase-, urease-, and catalase-positive motile bacteria. By Gram's staining the cells were recognized as curved rods, while Giemsa's stained cells were observed to be spiral rods. The fresh *H. pylori* cultures showed up as small, transparent, and convex colonies like dewdrops, which, with time,

changed to larger and translucent colonies. The identification was further confirmed by the established API indices. The API Campy index revealed a typical growth of *H. pylori* with index numbers 1001004, 1201004, and 5201004 having more than 80% conformity. The API numbers 2220004, 2301544, and 6021514 conformed to Campylocobacter other than *H. pylori*.

Anti-*H. pylori* activity (in vitro)

MICs of AMX, CLT, MNZ, TET, famotidine, zinc-famotidine complex, and a simple mixture of famotidine and zinc acetate

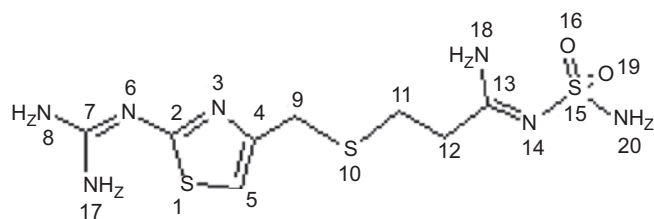


Figure 1. Numbering scheme of famotidine.

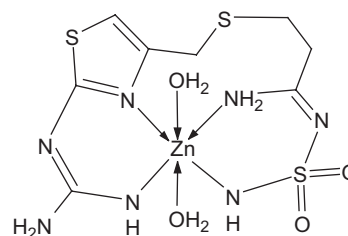


Figure 3. Proposed structure of the complex.

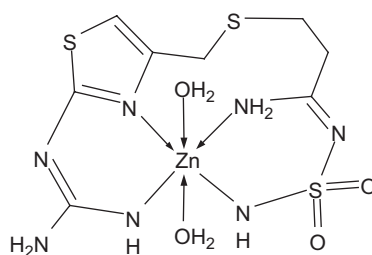
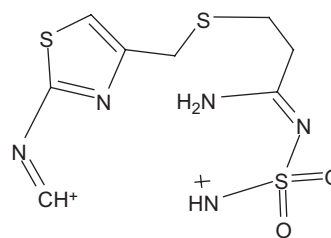
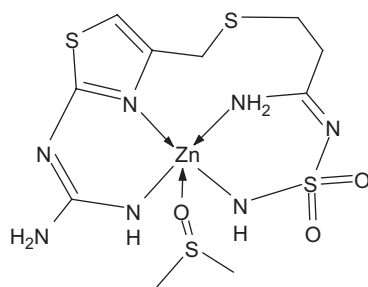
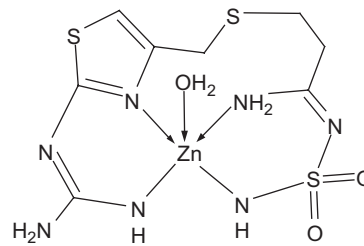
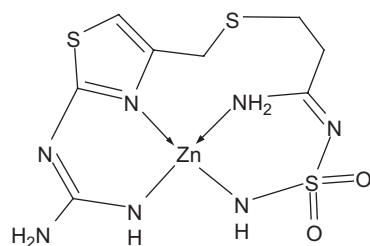


Figure 2. Fragmentation pattern of Zn(famotidine)-2H₂O (without washing).

Table 4. Minimum inhibitory concentrations ($\mu\text{g mL}^{-1}$) of the compounds under investigation against 34 clinical isolates and two reference strains of *H. pylori*.

<i>H. pylori</i> strains	AMX	CLT	TET	MNZ	Famotidine	Zn(famotidine) \cdot 2H ₂ O (without washing)	Mixture of famotidine and zinc acetate dihydrate
Reference strains							
NCTC-11637	0.5	0.5	2	16	32	1	64
NCTC-11638	0.125	0.5	2	4	64	1	64
Local isolates							
SA-1	0.125	0.25	16	32	64	2	64
SA-2	0.25	0.25	1	4	64	4	64
SA-3	0.125	1	32	64	64	4	64
SA-4	0.125	0.25	1	2	32	2	64
SA-5	0.25	4	32	16	32	4	64
SA-6	16	0.5	2	16	64	8	64
SA-7	8	0.25	4	32	64	1	64
SA-8	0.25	0.25	1	64	64	1	64
SA-9	0.125	0.5	0.5	128	64	1	64
SA-10	0.25	0.25	1	256	16	2	64
SA-11	0.25	0.25	0.25	32	32	8	64
SA-12	0.125	0.5	0.25	64	64	8	128
SA-13	0.5	0.125	2	16	64	8	128
SA-14	0.5	0.5	64	4	64	8	64
SA-15	0.25	0.125	8	256	64	2	64
SA-16	0.25	0.5	8	512	64	0.5	128
SA-17	4	8	8	512	64	0.5	64
SA-18	0.125	0.125	0.5	32	64	0.5	64
SA-19	0.25	0.125	0.25	32	64	0.5	64
SA-20	0.5	0.125	0.5	32	64	2	64
SA-21	0.25	0.5	32	64	64	2	128
SA-22	0.25	0.125	0.25	16	64	4	64
SA-23	0.25	0.125	0.5	8	128	4	64
SA-24	0.25	0.5	0.25	8	128	8	32
SA-25	0.25	0.125	0.5	8	128	1	64
SA-26	0.25	0.5	0.25	64	128	0.5	64
SA-27	0.5	0.125	0.25	64	64	0.5	128
SA-28	0.5	8	0.25	512	64	4	64
SA-29	0.125	0.5	0.25	512	64	4	32
SA-30	0.5	0.125	1	512	64	8	64
SA-31	8	0.5	1	256	64	8	64
SA-32	0.125	0.125	0.25	16	64	1	64
SA-33	0.125	0.125	1	16	64	1	128
SA-34	32	0.5	0.25	16	64	1	128

dihydrate against 34 clinical isolates and two reference strains of *H. pylori* are given in Table 4. The antibacterial activities of the complex against AMX-resistant clinical isolates (SA-6, 7, 17, 21, 31, and SA-34), CLT-resistant clinical isolates (SA-3, 4, 17, and SA-28), TET-resistant clinical isolates (SA-1, 3, 5, 14, and SA-21), and MNZ-resistant clinical isolates (SA-1, 3, 5–14, 16–22, and 6–34) were nearly comparable to those against AMX-, CLT-, TET-, and MNZ-susceptible isolates. The complex was also found to be equally effective against the strains showing double (SA-1, 6, 7, 21, 28, 31, and SA-34) and triple (SA-3, 5, and 17) drug resistance. With standard 7–14-day triple therapies, eradication of *H. pylori* is decreasing due to increased bacteria-resistant strains. The relevant resistance seems to be attributable to the types of antibiotic

prescribed in eradication therapy, especially MNZ, CLT, and AMX. Hence, there is a great medical need for the eradication of *H. pylori* by single therapy if possible. Bismuth is the only metal being widely used as an antibacterial agent against *H. pylori* as part of triple therapy. Due to the potential toxic effects of bismuth, dietary metals such as zinc, having well-defined antimicrobial activities, can be seen as possible treatments for gastrointestinal symptoms and infections³³. Zinc compounds have been reported as antiulcer agents in animals and humans. Since *H. pylori* is a major peptic ulcer-causing agent, the possibility has been raised that part of the antiulcer activity of zinc could be due to its effects on the growth of *H. pylori*. *In vitro* effects of zinc sulfate and a slow-release zinc compound, zinc monoglycerolate (ZMG), on

Table 5. Inhibition (% ± SD) of human urease by Zn(famotidine)·2H₂O (without washing).

Inhibition at						
8 μM	4 μM	2 μM	1 μM	0.5 μM	0.25 μM	0.125 μM
64.005 ± 1.063	32.134 ± 1.122	16.107 ± 0.525	8.865 ± 1.562	4.15 ± 1.435	2.134 ± 1.011	1.34 ± 1.109

Table 6. LD₅₀ values of famotidine and its complex.

Drug/complex	LD ₅₀ (mg kg ⁻¹)
Famotidine	4100
Zn(famotidine)·2H ₂ O	5950

the growth of *H. pylori* cultured in either solid agar or broth media showed that Zn²⁺ and ZMG alone had little effect on the growth of *H. pylori* in the solid or liquid phase. However, incorporating a ligand such as *O*-cyclodextrin (*O*-CyD) with zinc produced marked inhibition of growth in all *H. pylori* strains. It was argued that incorporation of *O*-CyD may aid the penetration of zinc compounds into bacteria. It has been proposed that in order to improve the antimicrobial efficacy of zinc, it should be chelated with ligands³⁴. In the present study, zinc was chelated with famotidine and tested against *H. pylori*. *In vitro* studies revealed that the zinc-famotidine complex, and not a simple mixture of zinc salt and famotidine, possessed activity. This was confirmed by taking the MICs of a mixture containing famotidine and the zinc salt.

Urease inhibitory activity

The complex under investigation was found to exhibit an inhibitory effect (Table 5) against human urease at all tested concentrations. Inhibition was found to be linear with concentration of the complex. Ureases obtained from different sources normally contain, in addition to the nickel metal, 1–3 protein subunits in varying stoichiometric ratios³⁵. A urease inhibitor may interact with either the metal or the protein component to interfere with the enzyme activity. A wide variety of mechanisms including competitive, non-competitive, or cooperative binding are known to be involved in the interaction of an inhibitor with an enzyme. The exact mechanism of urease inhibition by the test complex could not be determined. The compound could be thought of as undergoing ligand exchange reactions, causing the inhibition of enzyme activity.

Toxicity study

The LD₅₀ value of the complex under investigation is given in Table 6. The results indicate that the zinc complex possessed a substantially higher value as compared with that of the parent drug. Thus, the complex appears to be far less toxic than the parent drug.

Conclusions

A solvent-free synthesis route for the zinc(II)-famotidine complex has been developed, and the complex was characterized by use of various spectroscopic techniques. This work clearly demonstrates that solvent-free synthesis of the complex can be achieved. Based on the relatively higher

LD₅₀ value, anti-*H. pylori* activity, and urease inhibitory effect it can be concluded that complexation of famotidine with zinc can make the drug safer and more effective, for use as a single therapeutic agent against *H. pylori*.

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