Histamine H_2 Antagonists: Powerful Ligands for Copper(II). Reinterpretation of the Famotidine–Copper(II) System

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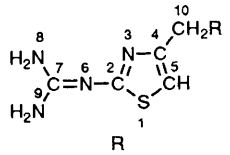
Potentiometric, absorption, EPR and ^{13}C NMR spectroscopic studies performed for a series of effective H_2 antagonists of histamine (imidazole-4-ethanamine) including effective antiulcer drug famotidine, have shown that all ligands containing a guanidine–thiazole fragment co-ordinate copper(II) ions at pH around 2 using two nitrogen donors. The guanidine moiety having acidic nitrogen (log *K* 1.5–3.0) acts as an anchor and the thiazole nitrogens with protonation constants around p*K* 6.7 very efficiently form a chelate ring. The adjacent thioether sulfur may also be involved in metal-ion binding, contributing to the stabilities of the complexes formed. At higher pH an amine terminal fragment is involved in co-ordination *via* one of its nitrogens leading to a {N,N,S,N} binding set. Comparison of all results obtained for the seven compounds studied strongly suggests that only equimolar species can be detected in this system. This allows a convincing reinterpretation of earlier studies on the copper(II)–famotidine system.

Famotidine, 3-{[2-(diaminomethyleneamino)thiazol-4-yl]-methylsulfanyl}-N^2-sulfamoylpropionamidine (L^1), is an efficient antiulcer drug having an excellent histamine (imidazole-4-ethanamine) H_2 receptor blocking effect, better than that of the earlier used cimetidine, *N*-cyano-*N'*-methyl-*N''*-{2-[(5-methyl-1*H*-imidazol-4-yl)methyl]sulfanylethyl}guanidine.[1] Our recent work on the binding ability of famotidine towards Cu^{II} has shown that this drug is extremely effective in co-ordination.[2,3] Copper(II) ions were shown to have a distinct impact on the chemistry and biochemistry of cimetidine,[4–6] although the computer-simulated distribution of the complexes involved does not show any important influence of this drug on the bioavailability of essential metal ions.[7,8] The binding ability of cimetidine is, however, much lower than that of famotidine. The complicated set of potential donors meant that it was possible to discuss the binding sites only tentatively. To obtain a clearer description of the copper(II)–famotidine system we have undertaken additional studies including several analogues of the drug. A set of histamine antagonists structurally related to famotidine was chosen to allow much more precise information to be obtained about the co-ordination equilibria, complex stoichiometry and stability constants.



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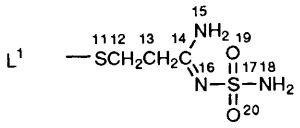
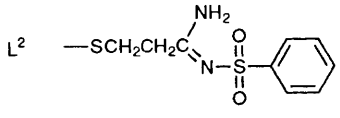
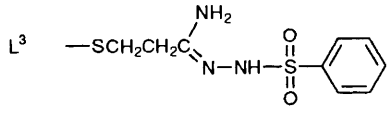


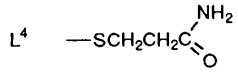
L^1



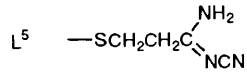
L^2



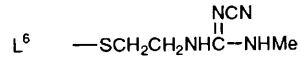
L^3



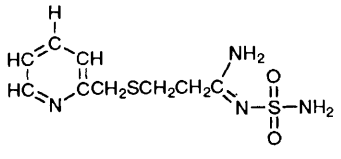
L^4



L^5



L^6



L^7

Experimental

Famotidine was a gift from Therapicon (Italy) used without further purification. Its purity was checked by potentiometric titration and HPLC. The derivatives L^2–L^5 and L^7 were synthesised as described earlier[9,10] and L^6 as described in ref. 11.

Spectroscopy.—The EPR spectra were recorded on a Radiometer SE/X spectrometer at X-band (9.3 GHz) at 120 K, in ethane-1,2-diol–water (1 : 2) as solvent, absorption spectra on a Beckman DU 650 spectrophotometer. Solutions containing 5×10^{-3} mol dm^{-3} Cu^{II} with metal-to-ligand molar ratios of 1 : 2 to 1 : 5 were used for spectroscopic measurements. Proton and ^{13}C NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer with $SiMe_4$ as a standard in Me_2SO (^{13}C) or D_2O (^1H).

Table 1 Proton dissociation constants (pK) of famotidine and derivatives at 25.0 °C and I = 0.1 mol dm^{-3} (KNO_3). Data for cimetidine are given for comparison

	log β				pK			
Compound	HL	H_2L	H_3L	H_4L	K_1	K_2	K_3	K_4
Famotidine (L^1)	11.12 ± 0.01	17.83 ± 0.01	19.31 ± 0.04	—	11.12	6.71	1.48	
L^6	11.61 ± 0.01	18.39 ± 0.01	20.49 ± 0.02	—	11.61	6.78	2.10	
L^3	10.04 ± 0.01	16.91 ± 0.01	21.92 ± 0.01	24.94 ± 0.01	10.04	6.87	3.02	5.01
L^4	6.88 ± 0.01	9.30 ± 0.01	—	—		6.88	2.42	
L^5	6.81 ± 0.01	9.00 ± 0.01	—	—		6.81	2.19	
L^2	6.84 ± 0.01	9.30 ± 0.01	—	—		6.84	2.46	
L^7	10.89 ± 0.01	15.26 ± 0.01	—	—	10.89	4.37		
Cimetidine *	6.70 ± 0.01							

Protonation constants: K_1 of amide/amidine terminal nitrogen; K_2 of thiazole or pyridine (L^7) nitrogen; K_3 of guanidine nitrogen [N(8) + N(9)]; K_4 of hydrazide nitrogen. * Data from ref. 7.

Potentiometry.—Stability constants for H^+ and copper(II) complexes were calculated from titration curves carried out at 25 °C using total volumes of 2.0 cm^3 and MOLSPIN automatic titration system. Changes in pH were followed by using a glass–calomel electrode (Russell CMAWL) calibrated for hydrogen-ion activity. The relationship between activity and concentration was calculated daily by titration with HNO_3.[12] All solutions were prepared in 0.1 mol dm^{-3} KNO_3; a concentration of 10^{-3} mol dm^{-3} Cu^{II} and metal-to-ligand ratios of 1:1, 1:2 and 1:4 were used. Three or four titrations were performed over the range pH 2–10.5 for the free proligands and over the range pH 2.5–10.5 for metal-containing systems with famotidine, L^4 and L^6, from pH 2.5 to 7.5 with L^2, L^5 and L^7 and from pH 2.5 to 6.0 with L^3 due to complex precipitation in more basic solutions. Stability constants $\beta_{pqr} = [M_pH_qL_r]/[M]_p[H]_q[L]_r$ were calculated with the aid of the SUPERQUAD computer program.[13] The standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species involved in the equilibria.

Results and Discussion

Proton Complexes.—Protonation constants for all compounds studied are given in Table 1. Careful evaluation of the titration curves at low (< 3) and high (> 10) pH allow the calculation of three protonation constants for famotidine and L^6, two for L^2, L^4, L^5 and L^7 and four for L^3. In our previous famotidine studies[2,3] we have calculated only one proton dissociation constant originating from the thiazole ring nitrogen, pK around 6.7. All compounds in Table 1 except L^7 are characterised by deprotonation of the thiazole nitrogen, with pK close to 6.8. However, detailed calculations of the titration curves performed in this work have shown that the compounds containing a guanidine moiety, *i.e.* famotidine, (L^1) and L^2–L^6, exhibit also one low pK value (1.5–3.0) which can be attributed to the guanidine residue (Table 1). The aromatic ring system of thiazole may be the cause of the considerable decrease in protonation constant of the guanidine moiety as its acidity is critically influenced by the substituent bound to the guanidine nitrogen.[14,15] Protonation of guanidine at low pH is also suggested by ^{13}C NMR spectra which indicate very distinct chemical shifts of the guanidine C(7) carbon when pH changes from 0.5 to 2.5 (Fig. 1). The splitting of the NMR spectra indicates an equilibrium between two famotidine isomers formed when guanidine is protonated. The distinct differences between the pK_3 values may be due to an interaction of the guanidine moiety with the amide terminal. This is likely for bent molecules as was seen in the crystal structure of famotidine.[16] The most effective impact on the guanidine proton dissociation is made by the strongly polar sulfonamide terminal of famotidine (Table 1).

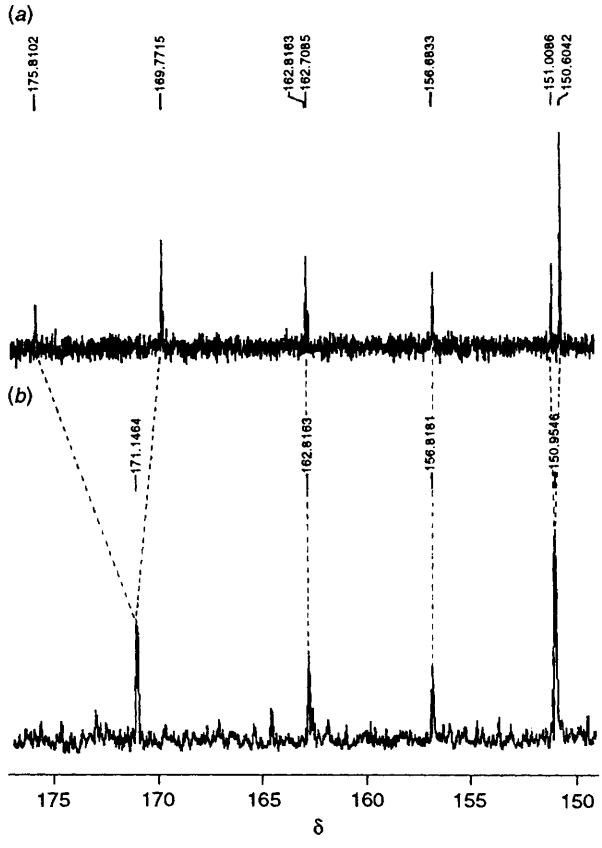


Fig. 1 Carbon-13 NMR spectra of famotidine (0.1 mol dm^{-3}) in D_2O at pH 0.49 (*a*) and 2.36 (*b*)

All the compounds studied differ in the terminal polar group which comprises various amide and amidine moieties. Four of the derivatives undergo protonation with log K between 10 and 11.5 (Table 1); L^3 having a hydrazide NHN unit also exhibits a pK value of 5.01 attributed to hydrazide nitrogen. All others (L^2, L^4, L^5) exhibit only two protonation constants which could be attributed to the guanidine and thiazole nitrogens. Compound L^7 is a pyridine derivative having pK 4.37 representing deprotonation of pyridine nitrogen and pK 10.89 assigned as above to the sulfonamide terminal nitrogen.

Copper(II) Complexes.—In earlier work on the copper(II)–famotidine system[2] the formation of 1:2 metal-to-ligand complexes was assumed, although unequivocal support for the co-ordination mode was difficult to obtain. To solve this problem we have chosen several compounds having exactly the same guanidine–thiazole–$CH_2SCH_2CH_2$ fragment and various

Table 2 Copper(II) complex formation constants (log β) with famotidine and its derivatives at 25.0 °C and I = 0.1 mol dm^{-3} (KNO_3). Data for cimetidine are given for comparison

Ligand	$M(H_2L)$	log K	M(HL)	log K
Famotidine (L^1)			17.15 ± 0.03	6.03[a]
L^6			18.21 ± 0.03	6.60[a]
L^3	21.41 ± 0.01	6.36[a]	18.13 ± 0.01	−3.28
L^4				
L^5				
L^2				
L^7			13.84 ± 0.01	2.92[a]
Cimetidine[b]				

Ligand	ML	pK	$MH_{-1}L$	pK	$MH_{-2}L$	pK
Famotidine (L^1)	10.83 ± 0.03	6.32	3.21 ± 0.03	7.62	−7.23 ± 0.04	10.44
L^6	11.87 ± 0.03	6.34	3.57 ± 0.03	8.30	−6.68 ± 0.09	10.25
L^3	12.19 ± 0.01	5.94				
L^4	6.45 ± 0.01		−0.35 ± 0.01	6.80	−9.01 ± 0.01	8.66
L^5	6.68 ± 0.01		0.32 ± 0.01	6.36	−7.21 ± 0.01	7.53
L^2	6.03 ± 0.01		−0.10 ± 0.01	6.13		
L^7	8.12 ± 0.01	5.72	0.56 ± 0.01	7.56		
Cimetidine[b]	4.16 ± 0.01					

Stepwise stability constants K for the reaction: $MH_nL \rightleftharpoons MH_{n-1}L + H^+$. [a] Stability constant for the first complex after subtraction of the protonation constant of the unbound nitrogen donor(s) (see text for L^3 and L^6). [b] Data from ref. 7.

amide/amidine terminals. One, L^7, possesses exactly the same sulfonamide fragment as that of famotidine but instead of the guanidine–thiazole moiety it has a simple pyridine ring system.

With L^3 *and* L^6. The most unambiguous calculations of the titration data were obtained for the copper(II)–L^3 and –L^6 systems. Only equimolar complex species could be fitted into experimental potentiometric titration curves (Table 2). The complex formation begins at pH around 2 and four or three complex species were detected in the pH range studied for L^6 and L^3, respectively [Table 2, Fig. 2(*a*)]. It is interesting that when protonation constants are taken into account (*i.e.* subtracted from the log β value of the respective complex) for both ligands, the stabilities of the first complexes formed, Cu(HL) (where HL is neutral) and $Cu(H_2L)$ (where H_2L is neutral) for L^6 and L^3, respectively are very similar (log K^* = 6.60 and 6.36, respectively Table 2). This suggests the same co-ordination mode in each case. According to spectroscopic data (Table 3), the d–d transition energy around 630 nm and EPR parameters suggest the involvement of two nitrogen donors in the metal-ion binding.[17] The low concentration of the $Cu(H_2L)$ species for the Cu^{II}–L^6 system does not allow recording of the EPR spectrum and its absorption band is much weaker than that of Cu(HL) of L^6 [Table 3, Fig. 2(*b*)]. Another band is observed around 400 nm indicating formation of a Cu^{II}–S bond.[2,18] The formation of consecutive complexes; CuL for L^6 and Cu(HL) for L^3 shifts the d–d transition towards higher energy (Table 3) indicating the involvement of the third nitrogen donor. The intensity of the S→Cu^{II} charge-transfer band around 400 nm considerably increases. Thus, both L^3 and L^6 seem to co-ordinate to Cu^{II} in the same way, starting most likely with the guanidine and thiazole nitrogens (see below). The formation of the chelate with N(9) [or N(8)] and N(3) nitrogen promotes some interaction with the adjacent thioether sulfur donor atom forming, at least partly, a second chelate and the {N,N,S} co-ordination mode. This mode corresponds well to the stability constants log K^* (Table 2) for Cu(HL) and $Cu(H_2L)$ species with L^6 and L^3, respectively. The third nitrogen involved is that of the amide terminal of the ligands. In the case of L^3 the formation of Cu(HL) involves the hydrazone nitrogen [log K = −3.28 for $Cu(H_2L) \longrightarrow Cu(HL) + H^+$],

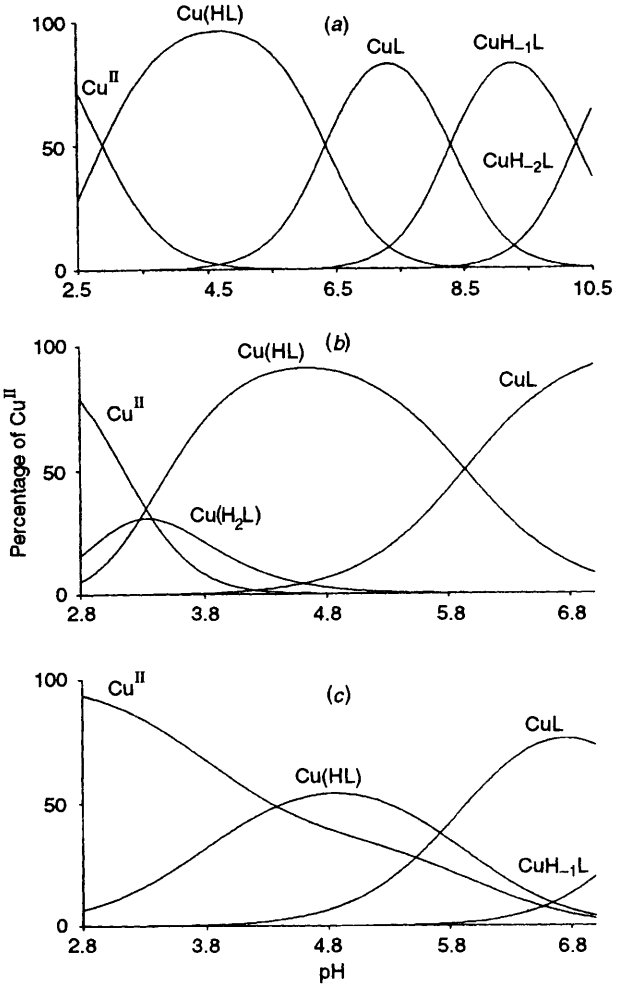


Fig. 2 Species distribution for copper(II)–L^6 (*a*), –L^3 (*b*) and –L^7 (*c*) systems at 1 : 2 metal-to-ligand ratio, I = 0.1 mol dm^{-3} at 25 °C, ligand concentration 3 × 10^{-3} mol dm^{-3}

Table 3 Spectral parameters (ESR, visible) for copper(II) complexes of famotidine, and its derivatives at 25.0 °C and I = 0.1 mol dm^{-3} (KNO_3)

Ligand	Species	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	$A_{\parallel}$/G	$g_{\parallel}$	Proposed co-ordination mode
Famotidine	M(HL)	4.5	636/413[a]	143/68	180	2.221	{N^8, N^3, S^{11}}
(L^1)	ML	7.5	604/438	224/162	177	2.217	{N^8, N^3, S^{11}, N^x}[b]
	$MH_{-1}L$	9.0	585/437	297/279	193	2.195	
	$MH_{-2}L$	10.3	574/435	310/309	176	2.220	
L^6	M(HL)	4.8	631/404	141/94	181	2.221	{N^8, N^3, S^{11}}
	ML	7.0	607/451	149/113	184	2.198	{N^8, N^3, S^{11}, N^x}
	$MH_{-1}L$	9.2	599/433	147/222	172	2.218	
	$MH_{-2}L$	10.2	598/431	153/254	171	2.218	
L^3	$M(H_2L)$	3.5	632/401	21/27			{N^8, N^3, S^{11}}
	M(HL)	4.5	588/413	182/163	172	2.220	{N^8, N^3, S^{11}, N^x}
	ML	6.0	546/414	122/214	182	2.185	
L^4	ML	5.0	637/399	136/77	177	2.333	{N^8, N^3, S^{11}}
	$MH_{-1}L$	7.5	610/443	167/138	171	2.213	{N^8, N^3, S^{11}, N^x}
	$MH_{-2}L$	9.5	603/436	162/302	174	2.214	
L^5	ML	4.1	631/398	120/70	178	2.229	{N^8,N^3,S^{11}}
	$MH_{-1}L$	6.7	618/393	131/119	181	2.225	{N^8,N^3,S^{11},N^x}
	$MH_{-2}L$	7.2	605/423	146/87	169	2.231	
L^2	ML	4.5	636/401	136/86	179	2.231	{N^8,N^3,S^{11}}
	$MH_{-1}L$	6.4	634/408	151/99	179	2.228	{N^8,N^3,S^{11},N^x}
L^7	ML	4.4	723	15	178	2.193	
	$MH_{-1}L$	6.1	613	101	167	2.232	{N_{py},S^{11}}[c]
	$MH_{-2}L$	7.5	586	255	170	2.111	{N_{py},S^{11},N^x}[c]

[a] d–d Transition/S→Cu charge transfer transition. [b] N^x = One of amide terminal nitrogens. [c] No S→Cu charge-transfer transition was seen.

while in the CuL complex of L^6 it is one of the amide nitrogens [log K for Cu(HL) ⟶ CuL + H^+ is −6.34]. The formation of $CuH_{-1}L$ (pK = 8.30) and $CuH_{-2}L$ (pK = 10.25) complexes with L^6 could correspond to deprotonation of metal-bound water molecules.[19,20] The copper(II)–thioether sulfur bond is rather long[21] and at least one equatorial bound water is very likely.

A considerably different situation is seen in the case of the Cu^{II}–L^3 system. Proton dissociation from the Cu(HL) to give the CuL species is much easier (pK = 5.94). This may suggest that the weakly basic hydrazide nitrogen is substituted by the more basic adjacent amide nitrogen donor having pK = 10.04. It is interesting that the stabilities of the CuL complexes of L^3 and L^6 are very similar suggesting very similar binding modes (Table 2). Precipitation of the complex prevents study above pH 6.0.

With L^1. In our earlier studies on the copper(II)–famotidine (L^1) system the potentiometric data were evaluated by assuming the formation of a CuL_2 species at pH > 3 and the two extreme pK values, 1.48 and 11.12, were not taken into consideration. The studies performed for L^3 and L^6 (see above) having exactly the same guanidine–thiazole fragment strongly indicated the formation of equimolar complexes only. The same assumption can be made for the copper(II)–famotidine system and the set of complexes obtained from the SUPERQUAD calculations of the potentiometric curves is exactly the same as that for L^6 (Table 2). Since the protonation patterns obtained in this work for L^6 and famotidine are the same (*i.e.* HL is neutral) (Table 1) and the protonation constants are similar (Table 1) it is reasonable to assume the same metal-ion binding mode with both drugs. As it is seen from Table 2, the same co-ordination model is obtained for both ligands and the complex stability constants are similar. As discussed above the d–d transitions observed in the absorption spectra strongly suggest the involvement of two or three nitrogen donors (Table 3, see above). The low pK value observed for famotidine (and other ligands, Table 1) indicates protonation at the guanidine moiety, as shown by ^{13}C NMR spectroscopy. This may suggest that Cu^{II} is first co-ordinated to the drug molecule *via* the relatively acidic guanidine nitrogen and then the thiazole nitrogen closes the chelate ring leading to formation of a {N(9),N(3),S} co-ordinated complex. To get some information about this primary binding site we performed a ^{13}C NMR study of the copper(II)–famotidine system looking at the chemical shift and linewidth variation upon addition of paramagnetic Cu^{II} to a famotidine-containing solution. The addition of a small amount of $CuCl_2$ to famotidine (ratio 1 : 1000 to 1 : 100) resulted in selective carbon signal shifts and signal broadening both at 23 and 80 °C. At 23 °C the observed chemical shifts (broadening in Hz) were −1.527 (54.92), −0.271 (2.18), 0.073 (13.80) and 0.319 ppm (21.17) for C(2), C(7), C(4) and C(5), respectively. These preliminary results clearly indicate that the anchoring binding sites for Cu^{II} in famotidine are the nitrogens of the guanidine and thiazole moieties. No changes upon addition of Cu^{II} were observed for the other famotidine carbons (detailed NMR studies on famotidine and its metal complexes will be published elsewhere).

The spectroscopic data obtained for different ligand-to-metal molar ratios (0.1 : 1 to 5 : 1) for famotidine–copper(II) solutions at pH 7 strongly suggest that the equimolar species is most likely. The spectra in the d–d region and the molar absorption coefficient are optimal for a 1 : 1 molar ratio.

The behaviour of the S→Cu^{II} charge-transfer transition with variation of pH is similar for famotidine and the other systems (where observed) including L^6 (Table 3). Its intensity increases up to pH 9.5 where the maximum number of ligand donors is involved in metal-ion co-ordination (*i.e.* three nitrogens and sulfur). This behaviour can easily be explained assuming that the copper(II)–thioether sulfur bond is rather weak and long. Thus, in complexes formed at lower pH for all ligands except L^7 the chelate formation with {N(9),N(3)} causes the sulfur terminal binding site to be partly co-ordinated to Cu^{II}. When a third nitrogen *e.g.* N(15) or N(16) is involved it forces the sulfur to form a stable bond with the metal creating second and third chelate rings.[21] This type of co-ordination pattern, {N,S,N,N}, was found in the crystal structure of a copper(II) complex with an analogue of cimetidine, structurally related to the ligands discussed in this work.[22] It should be mentioned that, due to the longer sulfur–metal bond, this donor could be co-ordinated apically to tetragonal Cu^{II} or, as often happens, lead to formation of trigonal-bipyramidal complexes.[21] The formation of $CuH_{-1}L$ or $CuH_{-2}L$ species is a good indication that water is interacting with the metal ion. The pK value of 10.44 ($CuH_{-1}L$ ⟶ $CuH_{-2}L$ + H^+) is similar to that

reported for deprotonation of metal-bound water.[19,20] The pK of the $CuL \longrightarrow CuH_{-1}L + H^+$ reaction for famotidine is rather low, 7.62, and could correspond to a change in the bound nitrogen (see above for L^6). However, parallel proton dissociation from bound water cannot be excluded.

With L^2, L^4 *and* L^5. All three compounds have two protonation constants corresponding to the guanidine and thiazole nitrogens (Table 1). Similar complex-formation behaviours are found. The formation of CuL species (where L is neutral) with nitrogens of the guanidine–thiazole moiety leads to stability constants having similar values (log β_{110} = 6.03–6.68, Table 2). They are also very close to those obtained for the complexes of famotidine (6.03), L^6 (6.60) and L^3 (6.36). This clearly indicates the same co-ordination mode for all these compounds.

With L^7. Compound L^7 is the only one studied which does not contain guanidine and instead of thiazole it has a pyridine ring. However, it contains the same sulfonamide terminal as famotidine. Its co-ordination ability is completely different from that of famotidine or other guanidine-containing compounds [Table 2, Fig. 2(*c*)]. It forms rather weak CuL and $CuH_{-1}L$ species (where HL is neutral) which involve pyridine nitrogen as an anchor donor. Thus, also the data obtained for the Cu^{II}–L^7 system indicate clearly the involvement of guanidine nitrogen in the formation of the first copper(II) complexes in the famotidine and other systems discussed.

Conclusion

These studies on the series of analogues of histamine H_2 antagonists and the antiulcer drug famotidine allow us to clarify the co-ordination equilibria, stability constants and binding modes in the complexes formed. The involvement of the guanidine and thiazole nitrogens creates very efficient ligands for Cu^{II} which, as shown before,[3] could effectively compete with naturally occurring chelating agents like histidine or histamine, especially at low pH (*e.g.* in the stomach). All antiulcer drugs based on guanidine–thiazole moieties are much more effective ligands than is cimetidine, the main binding site of which is an imidazole nitrogen.

Acknowledgements

This work was financially supported by the University of Wroclaw and Polish State Committee for Scientific Research. We thank Dr. I. Yanagisawa from Yamanouchi Pharmaceuticals, Japan, for samples of the histamine antagonists used.

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Received 7*th March* 1995; *Paper* 5/01381J