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Crystal structure of a copper(II)–famotidine complex and solution studies of the Cu^{2+}–famotidine–histidine ternary system

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The crystal structure of the complex $[CuL][ClO_4]_2$ (L = 3-{[2-diaminomethyleneamino)thiazol-4-yl]methylsulfanyl}-N^2-sulfamoylpropionamidine, famotidine) has been determined. It provides a full description of the metal binding sites and reveals the impact of metal co-ordination on the conformation of the drug molecule. Even metal ion binding is not able to change some conformational features of famotidine, which could be an important factor biologically. Potentiometric and spectroscopic data were obtained for the ternary species in the Cu^{2+}–famotidine–histidine system and show that famotidine is a very competitive chelating agent even in the presence of the strongly co-ordinating amino acid.

Famotidine, 3-{[2-diaminomethyleneamino)thiazol-4-yl]-methylsulfanyl}-N^2-sulfamoylpropionamidine, 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-ylmethyl]sulfanylethyl}guanidine.[1] Our recent work on the binding ability of famotidine towards Cu^{2+} ions has shown that it is extremely effective in co-ordination.[2] Potentiometric, absorption, EPR and ^{13}C NMR spectroscopic studies performed for series of effective H_2 antagonists of histamine including famotidine have shown that all compounds containing a guanidine thiazole fragment co-ordinate Cu^{2+} ions at pH around 2 through two nitrogen donors. The guanidine moiety having an unusually acidic nitrogen (log *K* 1.5–3.0) acts as an anchor and the thiazole nitrogens with log protonation constants around 6.7 close the chelate ring very efficiently. 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 the formation of a N,N,S,N binding set. A comparison of the results obtained for seven different compounds strongly suggested that only equimolar species can be detected in this system and this allowed us to make a convincing reinterpretation of earlier studies on the copper(II)–famotidine system.

In order to elucidate the complete structure of the copper(II) complex we have made an X-ray study of it. Since famotidine may compete with natural ligands in metal-ion binding, *e.g.* in blood plasma, we have also studied the copper(II)–famotidine–histidine (His) ternary system where His as one of the most effective low-molecular-weight natural chelating agents.

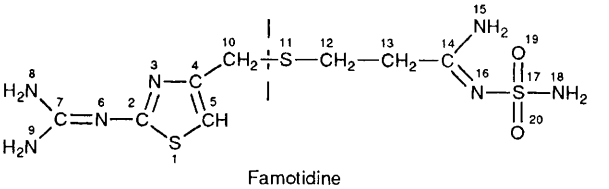
Experimental

Famotidine was a gift from Therapicon (Italy) used without further purification. Its purity was checked by potentiometric titration and HPLC. Histidine was used as obtained from Fluka.

Crystallography

Violet crystals of the complex were obtained from an aqueous solution containing $Cu(ClO_4)_2$ with slight excess of famotidine. Crystal data and details of the data collection and refinement procedure are given in Table 1, bond distances and angles in Table 2.

Intensity data were collected with a KM4 computer-controlled four-circle diffractometer.[3] The structure was solved by direct methods using the SHELXS 86 set of programs[4] and refined by full-matrix least-squares methods on F^2 using SHELXL 93[5] with anisotropic thermal parameters for non-hydrogen atoms. Corrections for Lorentz and polarisation effects were made (no absorption or extinction corrections were taken into account). All H atoms were located from successive Fourier-difference maps. The positional and thermal parameters of hydrogen atoms were refined.



Famotidine

Spectroscopic studies

The EPR spectra were recorded on a Bruker ESP 300E spectrometer at X-band (9.3 GHz) at 120 K, in ethane-1,2-diol–water (1:2) as a solvent, absorption spectra on a Beckman DU 650 spectrophotometer and circular dichroism spectra on a JASCO 600 spectropolarimeter. The concentrations used were similar to those given below for potentiometric titrations.

Potentiometric measurements

The stability constants for the Cu^{2+}–famotidine–His system were calculated from pH-titration data obtained at 25 °C with a MOLSPIN automatic titration system. Changes in pH were monitored by using a combined pH electrode (Russell CMAW 757) calibrated for H^+ activity. The relationship between activity and concentration was calculated daily by titration with HNO_3.[6] All solutions were prepared in 0.1 mol dm^{-3} KNO_3, using 0.003 mol dm^{-3} Cu^{2+} and a Cu^{2+} : famotidine : His molar ratio of 1:1:1. Two titrations were performed over the range pH 2.5–10.5 using volumes of 2.0 cm^3. Stability constants $\beta_{pqrs} = [M_pH_sL_q(L'_r)]/[M]^p[H]^s[L]^q[L']^r$ were calculated with the aid of the SUPERQUAD computer program.[7] The standard deviations reported were calculated by assuming random errors.

Results and Discussion

Structure of the complex $[CuL][ClO_4]_2$

As shown in Fig. 1 the crystal structure provides support for the co-ordination mode assumed in our earlier work based on

spectroscopic and potentiometric data.[2] The binding mode involves the guanidine and thiazole nitrogens, as well as the thioether sulfur and amidine terminal nitrogen, N(16). The X-ray data give much valuable information about the drug molecule conformation in its complexed form and allow one to compare precisely the structure of free and bound famotidine in order to discuss the structure–activity relationship. The crystal structure of metal-free famotidine shows various forms (polymorphism) depending on the conditions of crystallisation and the protonation state of the drug molecule.[8–10] Ishida *et al.*[10] discussed the possible stereostructure–activity relationship using X-ray data. The geometry observed in the crystal structures may not be representative of the actual conformation of the molecule bound to the H_2 receptor. However, in the absence of structural data for the receptor binding site one can consider at least the likely antagonist structures and their relations to biological activity.

The crystal structure of the present complex comprises $[CuL]^{2+}$ cations and anionic perchlorate groups. The binding of the neutral famotidine involves four donor atoms: thioether sulfur S(11), thiazole ring nitrogen N(3), guanidine nitrogen N(9) and amidine terminal nitrogen N(16) positioned around

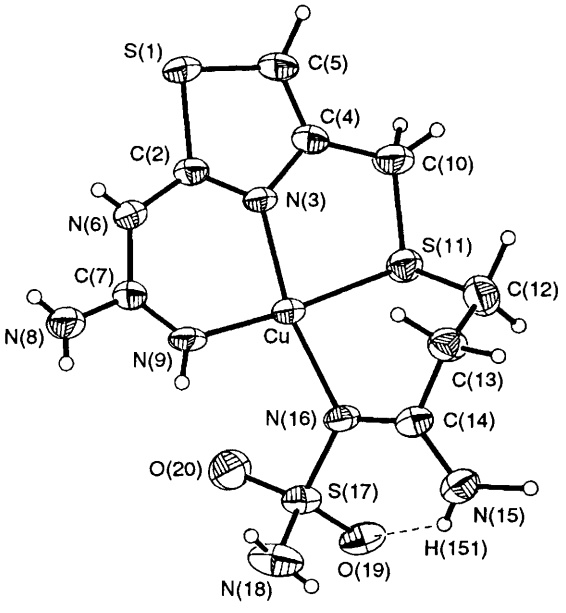


Fig. 1 Molecular structure of the complex $[CuL][ClO_4]_2$ showing the atom numbering and metal binding sites

Table 1 Crystal data and structure refinement for $[CuL][ClO_4]_2$

Empirical formula	$C_8H_{15}Cl_2CuN_7O_{10}S_3$
M	599.89
T/K	297(2)
λ(Cu-Kα)/Å	1.541 80
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	10.664(2)
b/Å	16.721(3)
c/Å	12.287(2)
β/°	112.77(3)
U/Å^3	2020.2(6)
Z	4
No. reflections for unit cell	25
θ Range for unit cell/°	24.5–38.6
D_c/Mg m^{-3}	1.972
D_m/Mg m^{-3}	1.98
μ/cm^{-1}	74.92
F(000)	1212
Crystal size/mm	0.15 × 0.15 × 0.18
Data collection method	ω–2θ
θ Range for data collection/°	4.50–81.98
No. of standard reflections every 100	3
hkl Ranges	−13 to 12, 0–21, 0–15
Independent reflections	3866
Data, restraints, parameters	3349, 0, 340
Goodness of fit on F^2	1.031
Final $R1$, $wR2$ [$I > 3\sigma(I)$]	0.0481, 0.1231
(all data)*	0.0481, 0.1231
Largest difference peak and hole/e Å^{-3}	0.993, −0.586

* $w = 1/[\sigma^2(F_o^2) + (0.0761P)^2 + 1.9217P]$ where $P = \frac{1}{3}(F_o^2 + 2F_c^2)$.

Table 2 Interatomic distances (Å) and angles (°) with estimated standard deviations in parentheses

Cu–N(3)	1.940(3)	Cu–S(11)	2.347(1)	N(9)–Cu–N(3)	89.6(1)	N(9)–Cu–S(11)	174.3(1)
Cu–N(9)	1.926(3)	Cu · · · O(14)	3.040(6)	N(9)–Cu–N(16)	97.3(1)	N(3)–Cu–S(11)	85.5(1)
Cu–N(16)	2.010(3)	Cu · · · O(20)	3.044(3)	N(3)–Cu–N(16)	164.8(1)	N(16)–Cu–S(11)	88.1(1)

	$[CuL][ClO_4]_2$	L·HCl[10]	L[9]		$[CuL][ClO_4]_2$	L·HCl[10]	L[9]
S(1)–C(2)	1.720(3)	1.720(5)	1.758(1)	C(10)–S(11)	1.831(4)	1.804(5)	1.832(1)
S(1)–C(5)	1.721(4)	1.717(6)	1.726(1)	S(11)–C(12)	1.822(4)	1.826(5)	1.806(1)
C(2)–N(3)	1.297(4)	1.279(6)	1.317(1)	C(12)–C(13)	1.541(6)	1.513(7)	1.535(2)
C(2)–N(6)	1.370(4)	1.403(6)	1.356(2)	C(13)–C(14)	1.494(5)	1.490(6)	1.506(1)
N(3)–C(4)	1.399(4)	1.404(6)	1.386(2)	C(14)–N(15)	1.316(5)	1.332(6)	1.312(1)
C(4)–C(5)	1.333(5)	1.331(7)	1.357(2)	C(14)–N(16)	1.328(5)	1.295(5)	1.323(1)
C(4)–C(10)	1.488(6)	1.494(7)	1.489(2)	N(16)–S(17)	1.665(3)	1.627(4)	1.612(1)
N(6)–C(7)	1.370(4)	1.370(6)	1.335(2)	S(17)–N(18)	1.602(4)	1.619(4)	1.628(1)
C(7)–N(8)	1.329(5)	1.356(6)	1.339(2)	S(17)–O(19)	1.434(3)	1.439(3)	1.440(1)
C(7)–N(9)	1.286(5)	1.278(6)	1.333(1)	S(17)–O(20)	1.419(3)	1.440(3)	1.445(1)
C(7)–N(9)–Cu	130.8(2)			C(4)–C(10)–S(11)	111.8(3)	113.8(2)	113.4(1)
C(2)–S(1)–C(5)	88.9(2)	87.3(2)	89.6(1)	C(10)–S(11)–C(12)	103.0(3)	103.4(2)	101.9(1)
S(1)–C(2)–N(3)	114.6(2)	116.9(2)	113.3(1)	S(11)–C(12)–C(13)	113.0(3)	111.9(2)	114.9(1)
S(1)–C(2)–N(6)	120.1(3)	117.1(2)	116.5(1)	C(12)–C(13)–C(14)	109.5(3)	114.1(3)	111.8(1)
N(3)–C(2)–N(6)	125.2(3)	126.0(3)	130.2(1)	C(13)–C(14)–N(15)	117.2(4)	116.3(3)	117.1(1)
C(2)–N(3)–C(4)	111.1(3)	109.8(3)	111.3(1)	C(13)–C(14)–N(16)	116.4(3)	115.9(3)	115.3(1)
N(3)–C(4)–C(5)	114.2(3)	113.8(3)	115.8(1)	N(15)–C(14)–N(16)	126.4(3)	127.8(3)	127.6(1)
N(3)–C(4)–C(10)	119.3(3)	118.3(3)	118.3(1)	C(14)–N(16)–S(17)	120.6(2)	121.3(2)	120.8(1)
C(5)–C(4)–C(10)	126.4(3)	127.9(3)	125.9(1)	N(16)–S(17)–N(18)	108.8(2)	110.4(2)	106.0(1)
S(1)–C(5)–C(4)	111.2(3)	112.2(2)	110.1(1)	N(16)–S(17)–O(19)	108.4(2)	103.9(2)	104.7(1)
C(2)–N(6)–C(7)	125.7(3)	123.4(3)	120.1(1)	N(16)–S(17)–O(20)	103.2(2)	112.3(2)	111.8(1)
N(6)–C(7)–N(8)	114.1(3)	115.4(3)	117.5(1)	N(18)–S(17)–O(19)	106.5(2)	107.0(2)	105.5(1)
N(6)–C(7)–N(9)	121.5(3)	123.0(3)	124.9(1)	N(18)–S(17)–O(20)	108.6(3)	106.1(2)	111.1(1)
N(8)–C(7)–N(9)	124.4(3)	121.5(3)	117.6(1)	O(19)–S(17)–O(20)	120.8(2)	117.0(2)	117.3(1)

Table 3 Conformation of famotidine (L) (torsion angles/°) for the co-ordinated and free drug

Compound	ω_1 N(3)–C(4)–C(10)–S(11)	ω_2 C(4)–C(10)–S(11)–C(12)	ω_3 C(10)–S(11)–C(12)–C(13)	ω_4 S(11)–C(12)–C(13)–C(14)	ω_5 C(12)–C(13)–C(14)–N(15)
$[CuL][ClO_4]_2$	17.6(6)	−118.0(4)	107.0(4)	64.2(4)	99.5(4)
L·HCl	68.8(3)	62.5(3)	84.8(4)	−178.1(4)	−67.4(4)
L	56.8	62.7	−170.6	68.1	−129.8

Table 4 Spectral parameters (ESR, visible, CD) for copper(II) complexes of famotidine and histidine and for ternary complexes of Cu^{II}–famotidine–histidine at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

		UV/VIS		ESR		CD	
Ligand and species	pH	λ_{max}/nm	ε_{max}/dm^3 mol^{-1} cm^{-1}	$A_{\parallel}$G	$g_{\parallel}$	λ/nm	$\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}
Famotidine							
MHL							
1 1 1	4.5	636/413[a]	143/68	180	2.221		
1 0 1	7.5	604/438	224/162	177	2.217		
1 −1 1	9.0	585/437	297/279	193	2.195		
1 −2 1	10.3	574/435	310/309	176	2.220		
Histidine							
MHL′							
1 0 1	5.0	623/296	76/80			707[b]	−0.355
						335[c]	−0.067
1 0 2	7.0	640	118	178	2.224	695[b]	+0.702
						331[c]	−0.090
Famotidine–histidine							
MHL(L)							
1 1 1 1	7.5	609/444	136/63	176	2.249		
1 0 1 1	9.4	603/451	146/93	183	2.243	683[b]	+0.160
						313[c]	−0.581
						291[c]	+0.188
1 −1 1 1	10.5	600/442	145/96	180	2.247		

[a] d–d Transition/charge transfer S→Cu. [b] d–d Transition. [c] Overlapped N→Cu^{2+} charge transfer.

the metal ion in a nearly planar geometry. Atoms N(3) and N(16) and N(9) and S(11) are *trans* to each other, with angles N(3)–Cu–N(16) 164.8(1)° and N(9)–Cu–S(11) 174.3(1)°, respectively. The Cu–N bonds are rather short [1.940(3), 1.926(3) and 2.010(3) Å] and similar to those obtained for the cimetidine analogue.[11] Also the Cu–S(11) distance of 2.347(1) Å is short compared to metal–thioether sulfur bond lengths.[12,13] The short copper–thioether S bond is stabilised most likely by neighbouring strongly bound nitrogen donors which form two effective chelate rings.

The co-ordination plane through the four donor atoms N(3),N(9),N(16),S(11) is distinctly distorted; S(11) is 0.579(6) Å out of the three-nitrogen plane, while copper ion is 0.218(2) Å out of this plane. The C(2)–N(6) and N(6)–C(7) bond lengths in the guanidine moiety (Table 2) are longer in metal-bound famotidine than those in the free drug molecule and they are close to the distances found for its hydrochloride, which similarly to metal-bound famotidine has a protonated guanidine N(6) atom. The other two C–N bonds, C(7)–N(8) and C(7)–N(9), are distinctly shorter than the two mentioned above (Table 2). These bond lengths indicate, however, that also in co-ordinated guanidine the C–N bonds show nearly equal double-bond character as is the case in the metal-free compound. Thus, the conformation around C(2)–N(6)–C(7) is fixed because of coupling of the double-bond systems of guanidine and the thiazole ring. Also the angles C(2)–N(6)–C(7) close to 120° (Table 2) indicate the sp^2 hybridisation and double-bond character between the atoms involved. The metal co-ordination to N(16) leads to lengthening of the N(16)–S(17) bond distance from 1.612(1) Å for free famotidine to 1.665(3) for the metal-bound form (Table 2). The sulfamoyl group is in the form of a slightly distorted tetrahedron. The dashed line in Fig. 1 indicates an intramolecular hydrogen bond. Selected torsion angles are collected in Table 3. The earlier assumption of Ishida *et al.*[10] was that the combination of ω_3–ω_5 torsion angles could be closely related with the H_2-receptor antagonist activity. The hydrochloride form of the drug (L·HCl) with its extended conformation differs considerably from the folded conformation of the free drug. Upon metal co-ordination the conformation changes drastically (Table 3). Only torsion angle ω_4 involving the S(11)–C(12)–C(13)–(14) bond system remains only slightly changed when copper or H^+ (L·HCl) is bound to famotidine. This conformation results in the envelope-like conformation of the chelate ring involving S(11) and N(16) (Fig. 2) and could be critical for the antagonist activity of famotidine. The metal binding also has a slight effect on the structure of the guanidine thiazole moiety due to strong delocalisation of the double bond system in this part of famotidine.

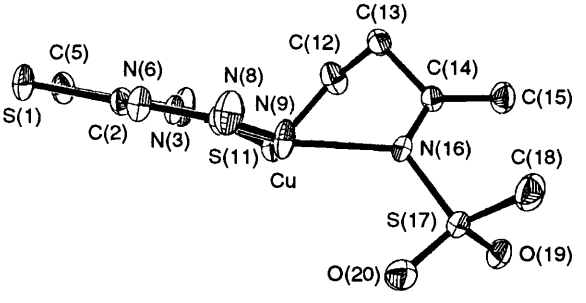


Fig. 2 Perspective view of the complex showing the structure of the drug in relation to the planar thiazole guanidine fragment

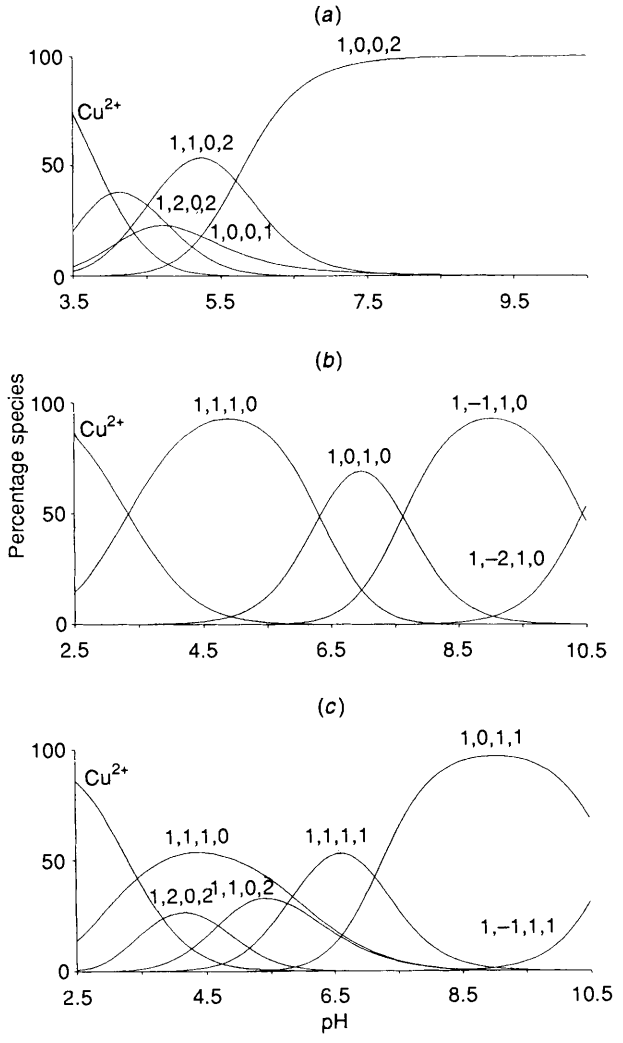


Fig. 3 Species distributions for Cu^{2+}–His (*a*), Cu^{2+}–famotidine (*b*) and ternary Cu^{2+}–famotidine–His (*c*) systems at ligand-to-metal molar ratios of 2:1 for (*a*) and (*b*) and 1:1:1 for (*c*). Concentration of Cu^{2+} 0.002 mol dm^{-3}. Complex notation is MHL(L') where L = famotidine and L' = His

Cu^{2+}-famotidine-His ternary systems

Our earlier study on this system showed that famotidine is a very competitive chelating agent for Cu^{2+} when compared to such natural ligands as histidine and histamine.[14] The recent data obtained for the Cu^{2+}–famotidine system have shown that only equimolar complexes[2] (see above) are formed with complete co-ordination to equatorial binding sites (Fig. 1) *via* a very effective N(3),N(9),N(16),S(11) donor set. This binding mode does not allow a second molecule to be co-ordinated. The addition of His to the Cu^{2+}–famotidine system, however, changes considerably the spectroscopic parameters suggesting the formation of a ternary system (Table 4). Potentiometric data obtained for equimolar solutions (Cu^{2+}:famotidine:His = 1:1:1) (Table 5, Fig. 3) can be fitted by three ternary complexes at pH > 4.5. At lower pH the [Cu(HL)] (L = famotidine) species is observed as a major complex due to very effective metal co-ordination to the guanidine and thiazole nitrogens. The protonated Cu^{2+}–His complex is also seen (Fig. 3). At pH > 5 the ternary complexes are the only major species observed. The spectroscopic parameters of the ternary complexes are distinctly different from those of the respective binary species (Table 4). On the other hand, the appearance of the CD spectrum in the d–d and charge-transfer regions when

Table 5 Stability constants (log β) for binary and ternary complexes formed in Cu^{II}–famotidine–histidine solutions at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

Ligand and species	log β
Famotidine	
MHL	
0 1 1	11.12 ± 0.01
0 2 1	17.83 ± 0.01
0 3 1	19.31 ± 0.04
1 1 1	17.15 ± 0.03
1 0 1	10.83 ± 0.03
1 −1 1	3.21 ± 0.03
1 −2 1	−7.23 ± 0.04
Histidine	
MHL'	
0 1 1	9.14 ± 0.01
0 2 1	15.17 ± 0.01
0 3 1	17.09 ± 0.01
1 0 1	9.58 ± 0.03
1 0 2	17.87 ± 0.01
1 1 2	23.58 ± 0.01
1 2 2	28.09 ± 0.01
Famotidine–histidine	
MHL(L')	
1 1 1 1	25.09 ± 0.02
1 0 1 1	17.96 ± 0.02
1 −1 1 1	7.12 ± 0.03

His is added to the optically inactive Cu^{2+}–famotidine system clearly indicates binding of the amino acid to the metal ion.

Acknowledgements

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