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Novel class of Bi(iii) hydroxamato complexes: synthesis, urease inhibitory activity and activity against H. pylori.

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Reaction of $Bi(NO_3)_3$ with benzohydroxamic acid (Bha) and salicylhydroxamic acid (Sha) gives the novel Bi(III) complexes $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ (**1**) and $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)(OH_2)_2(Sha_{-1H})_{12}](NO_3)_2$ (**2**)**.** X-ray crystal structure of **1** reveals two hydroxamato coordination modes; bidentate bridging (O, O') and bidentate non-bridging (O, O') and of **2** reveals one coordination mode; bidentate bridging (O, O'). **1,** specifically designed to and demonstrated to inhibit the activity of urease, exhibits excellent antibacterial activity against three strains of *Helicobacter pylori* with MIC ≥ 16 μg/mL.

Introduction,

Helicobacter pylori (*H. pylori*) is a microaerophilic and neutralophilic Gram-negative bacterial pathogen that can colonise the human stomach. *H. pylori* infection can cause dyspepsia, peptic ulcer disease and gastric cancer, the second most common cause of cancer death in the world.[1] The WHO classified *H. pylori* as a Class I Carcinogen in 1994 and significantly eradication of *H. pylori* infection reduces the incidence of gastric cancer.[2] More recently, *H. pylori* has been linked with extragastric diseases such as cardiovascular disease, diabetes mellitus, sideropenic anemia and gallbladder cancer for example.[3]

H. pylori has developed a number of ways to survive and colonise the harsh acidic environment of the gastric mucosa and in turn induce chronic infection. One of these mechanisms is "acid acclimation" whereby in the acidic environment of the stomach, periplasmic pH is adjusted by regulation of urease, UreI, and α-carbonic anhydrase.[4] Urease, a dinuclear nickel(II) enzyme, catalyses the conversion of urea into ammonia and carbon dioxide.[4] Upon a decrease in pH, UreI, a pH-gated urea channel in the cytoplasmic membrane, opens allowing urea to move into the cytoplasm. A concurrent increase in bacterial urease activity, produces the ammonia required to neutralise protons entering the cytoplasm and maintain the pH microenvironment at c. 6.8.[4] Urease, which accounts for 10–15% of total protein by weight,[5] can be considered to be *H. pylori's* achilles heel as its activity is vital to *H. pylori's* ability to survive and colonise the harsh acidic environment of the gastric mucosa and in turn induce chronic infection.[4]

A standard triple drug treatment (triple therapy) of two antibiotics in addition to a proton pump inhibitor (PPI) is the first-line treatment for *H.* pylori.[6] Significantly though, *H. pylori* infections are becoming increasingly difficult to eradicate due to antibiotic resistance. Antibacterial drug resistance is a serious global health problem and the prospect of untreatable bacterial infections is becoming a reality. *H. pylori* resistance rates for example in Europe and Asia for clarithromycin are 17.5 and 21.5% and for metronidazole are 34.9% and 95.4% respectively.[7, 8]

Consequently bismuth (Bi)-containing quadruple therapies are increasingly recommended as the first-line treatment in a number of countries.[6, 9, 10] Bismuth subsalicylate (BSS), colloidal bismuth subcitrate (CBS) and the more recently developed ranitidine bismuth citrate (RBS), are administered however without an exact understanding of their structure,[11, 12] behaviour in biological environment or indeed their mechanisms of action.[13] Recently innovative metallomic and metalloproteomic approaches have aided identification of Bi targeting proteins in *H. pylori* such as HspA, Ef-Tu, NapA and urease (UreA and UreB) and revealed that Bi(III) has a high selectivity for cysteines and histidines, particularly motif patterns of CX_nC, CX_nH and HX_nH. Evidence therefore suggests that Bi interference with metal homeostasis and oxidation reduction processes may be important.[14-18]

Significantly it was recently demonstrated that glutathione and multidrug resistance protein transporter remove Bi from human cells, thereby protecting the human body from associated toxicity and also providing a level of selectivity between human cells and bacterial cells that lack glutathione such as *H. pylori.*[19]

Thus there is renewed interest in the development of novel Bi-based drugs and investigation of their mode of action.[13, 20-25]

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Hydroxamic acids, a family of important bioligands, of general formula RCONR'OH, are well-known metal chelators, metalloenzyme inhibitors and NO donors, Figure 1.[26, 27]

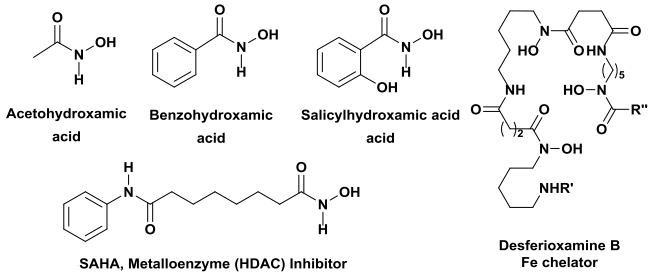


Figure 1 Representative structures of hydroxamic acids.

Significantly acetohydroxamic acid and alternative hydroxamic acids are potent inhibitors of urease. Acetohydroxamic acid for example has been demonstrated to bridge the dinuclear Ni(II) active site of the urease enzyme.[5]

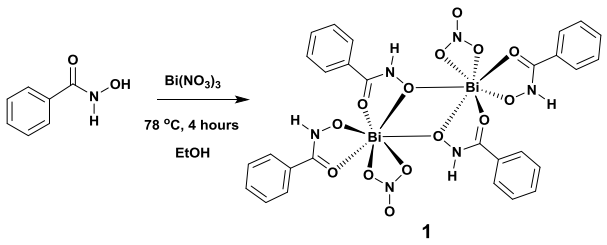
We therefore sought to combine the established antibacterial properties of Bi with the urease inhibitory properties of hydroxamic acids and develop structurally well-defined Bi hydroxamic acid complexes as potential anti-*H. pylori* agents. We hypothesise that such complexes will hydrolyse in the low pH of gastric juice releasing insoluble Bi salts such as BiOCl and free hydroxamic acids, i.e. co-deliver Bi and hydroxamic acids and activate at the site of action. In the gastric environment Bi salts and/or Bi gastrointestinal biomolecular complexes will exhibit conventional antibacterial activity against *H. pylori*. In addition free hydroxamic acids will inhibit the activity of *H. pylori* urease drastically reducing its ability to buffer the pH of its microenvironment.

Results and discussion

Synthesis

Reaction of hydroxamic acids (L) with $Bi(NO_3)_3$ in ethanol gave novel complexes of type $Bi(L_{-1H})_2NO_3$, representing a novel class of Bi hydroxamato complexes. Andrews *et al.* recently reported Bi hydroximato/ hydroxamato complexes on reaction of hydroxamic acids with $Bi(O^tBu)_3$ or $BiPh_3$.[24, 25]

Reaction of benzohydroxamic acid (Bha) with $Bi(NO_3)_3$ in absolute ethanol under reflux for 5 hours afforded the novel dinuclear Bi(III) complex $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ (**1**), Scheme 1. Crystals suitable for single crystal X-ray diffraction were obtained of **1** from recrystallization in methanol.



Scheme 1 Synthesis of Bi hydroxamato complex $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ (**1**).

In the ^{1}H NMR spectra (d_4-MeOD) of **1** for example complex no signals associated with the hydroxamic acid protons (N-H and O-H) are observed as expected. In **1** the most significant shift is that of an aromatic doublet, associated with protons closest to the hydroxamic acid group at the 2 and 6 positions on the aromatic ring, from 7.73 in the free ligand to 7.84.

The IR spectra of **1** exhibits distinctive ν(C=O) at 1595 cm^{-1} and the characteristic shift associated with (*O,O'*) hydroxamato coordination when compared to the corresponding ν(C=O) of the uncoordinated ligands (Bha, 1647 cm^{-1}).[28]

ESI-MS in the positive mode was used to identify product ion fragments associated with $BiNO_3$ (271.8) $Bi(Bha_{-1H})$ (345.7), $Bi(NO_3)(Bha_{-1H})$ (407.7), $Bi(Bha_{-1H})_2$ (480.7) for **1**.

Reaction of salicylhydroxamic acid (Sha) with $Bi(NO_3)_3$ in absolute ethanol under reflux for 5 hours afforded a Bi(III) complex of general formula $[Bi(NO_3)(Sha_{-1H})_2]_n$, which upon recrystallisation from methanol gave $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)_2(OH_2)_2(Sha_{-1H})_{12}].(CH_3OH)_4.(H_2O)_4$ **2**.

X-ray Crystallography

The solid state structure of **1** and **2** are shown in Figure 2. Both complexes crystallize in the $P\bar{1}$ space group and are completed by inversion symmetry.

The formula of **1** consists of the charge neutral complex $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ with a solvent MeOH molecule. The hydroxamato ligands and MeOH molecule display disorder with each phenyl ring occupying two positions and the solvent spread over six positions. In the main core of the complex each Bi centre is chelated by two ligands via Bi-O bonds (Bi1-O1 2.201(5); Bi1-O4 2.409(5)Å and Bi1-O11 2.234(4); Bi1-O14 2.276(5)Å) and a nitrato group (Bi1-O19 2.519(5); Bi1-O22 2.755(5)Å). The bismuth atoms are linked by two μ-oxo bridges via the benzohydroxamato groups forming a planar Bi_2-μ-O_2 dimer oxygen (Bi1-O11 2.234(4); Bi1-O11a$^{\$1}$ 2.674(5)Å; Bi-O-Bi$^{\$1}$ 113.23(19)°; $1 = symmetry transformation = -x+1,-y+1,-z+1). Significantly **1** exhibits two distinct and Bi hydroxamato binding modes: bidentate bridging (O, O') and bidentate non-bridging (O, O'), Scheme 1 and Figure 2.

Andrews and coworkers recently published the first X-ray crystal structure of a Bi hydroxamato complex; a unique Bi_{34} oxido-cluster, $[Bi_{34}O_{22}(Bha_{-2H})_{22}(Bha_{-1H})_{14}(DMSO)_6]$, isolated on dissolution of a $[Bi_2(Bha_{-2H})_3]$ complex in dmso/toluene. This complex features both doubly deprotonated benzohydroximato and singly deprotonated hydroxamato ligands.[24, 25]

The formula of **2** consists of the cationic complex $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)_2(OH_2)_2(Sha_{-1H})_{12}]^{2+}$ with two isolated NO_3^- counterions to balance the charge as well as solvent molecules (4 water and 4 methanol). The complex itself consists of 6 bismuth centres, each chelated by two salicylhydroxamato ligands. One terminal bismuth atom, Bi2, is bonded to a H_2O and methanol; the other terminal bismuth, Bi3, to a NO_3^- group. Each central Bi atom is also bonded to one NO_3^- group. The bismuth atoms are bridged by the salicylhydroxamato ligands to form terminal Bi_2-μ-O_3 (Bi-O range = 2.226-2.82Å, Bi-O-Bi range = 96.3(1)-101.4(1)°) and

planar central Bi_2-μ-O_2 dimers (Bi1-O21 2.337(2), O21-Bi1$^{\$1}$ 2.577(2)Å; Bi-O-Bi 117.1(1)°). The dimers are linked together by additional μ-O bonding via the amido hydroxy oxygen (O10-Bi1 2.256(2), O10-Bi3$^{\$3}$, 2.721(2)Å; Bi-O-Bi 121.6(1)°: O32-Bi1 2.521(2), O32-Bi2 2.230(2)Å; Bi-O-Bi 115.8(1)°: $3 = symmetry transformation = -x+1,-y+1,-z+1). In contrast to **1**, **2** exhibits only the bidentate bridging (O, O') hydroxamato coordination mode, Figure 2.

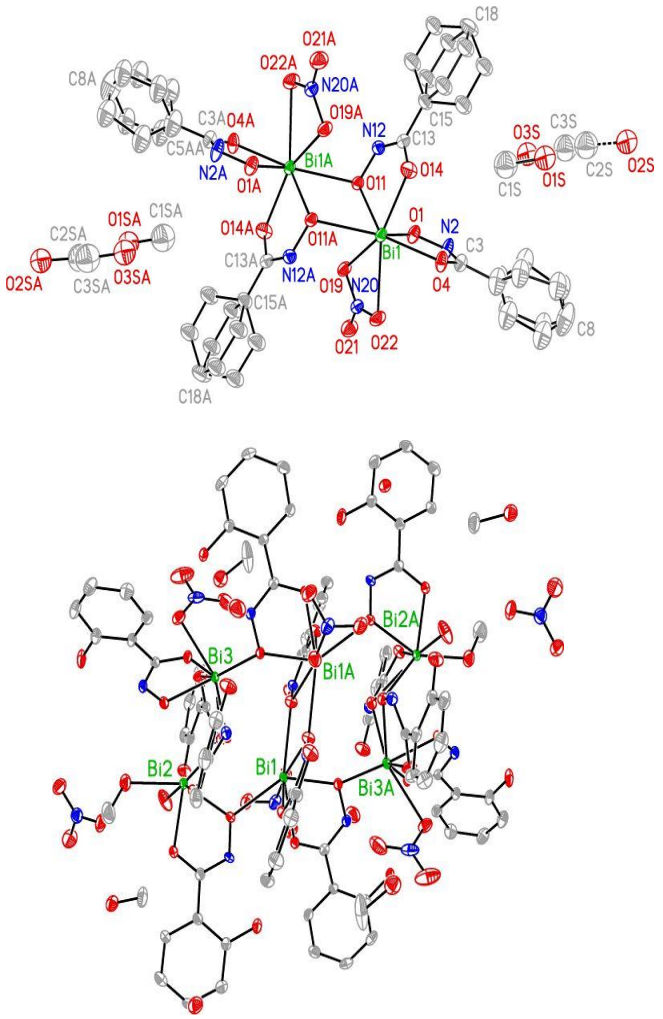


Figure 2 Molecular structure of $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2].CH_3OH$ (**1,** top, displacement ellipsoids shown at 50%, disordered phenyl carbon atoms and all hydrogen atoms omitted for clarity) and $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)(OH_2)_2(Sha_{-1H})_{12}](NO_3)_2.(CH_3OH)_4.(H_2O)_4$ (**2**,bottom, displacement ellipsoids shown at 50%, partial labelling and hydrogen atoms omitted for clarity).

Andrews *et. al.* recently reported the X-ray crystal structure of a Bi salicylhydroximato/hydroxamato dmso complex $\{[Bi(Sha_{-2H})(Sha_{-1H})(DMSO)_2][Bi(Sha_{-2H})(Sha_{-1H})(DMSO)]\cdot DMSO\}_\infty$.[25]
The coordination environment around each bismuth atom in **1** and **2** can be completed by including slightly longer O-Bi distances (Bi-O range complex 1 =2.2-3.03 Å; complex 2 = 2.2 – 3.2Å), Figure 3.
In complex **1**, this involves an intermolecular Bi-O interaction (Bi_O1$^{\$2}$, 3.034(5)Å; $2 = 1+x, y, z) and results in two 8 coordinate Bi atoms with edge shared distorted dodecahedral geometry. In complex **2**, Bi1 and Bi3 have a coordination number 9 with distorted monocapped square antiprismatic geometry, whereas Bi2 has a coordination of 8 with distorted dodecahedral geometry, Figure 3.

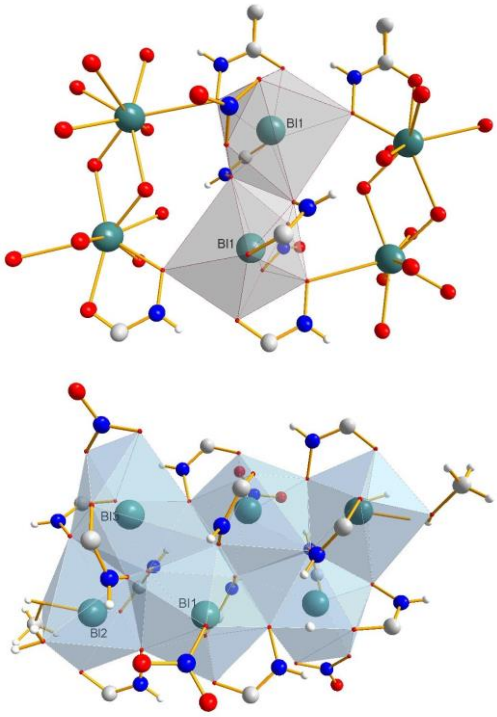


Figure 3. Polyhedral coordination environment around Bi in **1** (top) and 2 (bottom). Phenyl rings removed from ligands for clarity.

Biological Testing

Complex **1,** the first reported example of a Bi complex specifically designed to inhibit the activity of urease was selected to be tested for its urease inhibitory activity and activity against *H. pylori* given it is free of undesirable coordinated solvent molecules and therefore a suitable candidate as an anti *H. pylori* agent. Complex **2,** which contains coordinated MeOH molecules, is not a suitable drug candidate.

Urease Inhibitory Activity

Urease **1** and its corresponding hydroxamic acid ligand Bha

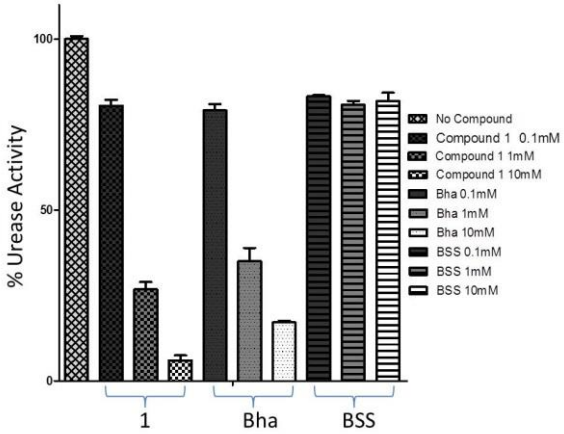


Figure 4 % Inhibition of the activity of Jack Bean Urease for compound **1**, Bha and BSS over the range 0.1-10 mM (n=3).

inhibited the activity of Jack Bean urease *in vitro* (e.g. 96 % inhibition and 83 % inhibition at 10 mM respectively), Figure 4. In contrast the clinically used Bi drug, BSS, showed poor inhibitory activity (19 %) even at 10 mM, Figure 4. The urease inhibitory activity of **1** is primarily attributed to the urease inhibitory properties of its benzohydroxamic ligand.

It is noteworthy that Sun *et al.* demonstrated both competitive and non-competitive inhibition of urease by Bi(III) using a number of complexes including RBC. Using NMR spectroscopic and site-directed mutagenesis studies they indicate that Bi(III) can bind to the highly conserved cysteine residue (Cys319) located at the entrance of the urease active site.[29] Therefore Bi can potentially also contribute to urease inhibition.

Antibacterial Activity

The minimum inhibitory concentrations (MICs) of compound **1** and its hydroxamic acid ligand Bha and BSS were determined against three laboratory strains of *H. pylori*: 26695, J99 and 60190 using the agar diffusion method (recommended by the Clinical and Laboratory Standards Institute, CLSI),[30] at compound concentrations ranging from 0.5 to 64 μg/mL. MICs of 16, μg/mL were determined for compound **1** against 26696, J99 and 60190, which were comparable to the MICs for BSS (16, μg/mL) and less than the MIC for the free ligand Bha, (32 μg/mL), which also exhibited considerable activity.

Table 1 MIC's for Complexes **1** against H. pylori (n=3).

H. pylori strain	1 (μg/ml)	Bha (μg/ml)	BSS (μg/ml)
26695	16	32	16
J99	16	32	16
60190	16	32	16

Andrews and coworkers recently reported alternative Bi(III) benzohydroxamato/hydroximato complexes; $[Bi_2(Bha_{-2H})_3]_\infty$, $[Bi(Bha_{-1H})_3]$ and $[Bi(Bha_{-2H})(Bha_{-1H})]$ with superior activity against *H. pylori* strains 26695 and 60190 with MIC's ranging from 0.09 to 1.56 μg/mL and likely due to superior *H. pylori* uptake.[24, 25]

Conclusions

Herein we report a novel class of Bi hydroxamato complexes of type $Bi(L_{-1H})_2.NO_3$ specifically designed to and demonstrated to inhibit urease, *H. pylori's* Achilles heel. Reaction of $Bi(NO_3)_3$ with benzohydroxamic acid (Bha) and salicylhydroxamic acid (Sha) gives the novel Bi(III) complexes $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ and $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)(OH_2)_2(Sha_{-1H})_{12}](NO_3)_2$. X-ray crystal structure of **1** reveals two hydroxamato coordination modes; bidentate bridging (O, O') and bidentate non-bridging (O, O') and of **2** reveals one coordination mode; bidentate bridging (O, O'). **1** exhibits excellent antibacterial activity against three strains of *H. pylori* with MIC ≥ 16 μg/mL.

Further development of bismuth complexes as effective urease inhibitors will be undertaken and their potential activity against *H. pylori* at low pH and increased urease dependent conditions investigated.

Experimental

Materials and instrumentation

Benzohydroxamic acid was purchased from Tokyo Chemical Industry Ltd. Salicylhydroxamic acid, Bismuth Subsalicylate, $Bi(NO_3)_3.5H_2O$ and deuterated solvents were purchased from Sigma Aldrich and used without further purification. IR spectra were recorded using a Bruker VERTEX 70/70v FT-IR spectrometer using KBr discs (4000-400 cm^{-1}). The spectra were analysed using OPUS software. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. The spectra were analysed using MestReNova software. The residual undeuterated d_4-Methanol signal at 3.31 ppm was used as an internal reference. Mass spectrometry experiments were performed on an Advion Expression Compact Mass Spectrometer where 10 μL of the samples were injected in 300 μL of methanol:water:formic acid (90:9:1 v/v). The mass spectrometry data were acquired both in positive and negative ion modes and the spectra analysed using the Advion Mass Express software programme.

Elemental analysis (C, H and N) was performed at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin, Ireland.

Isolated Jack bean (Canavalia ensiformis) urease and Fetal Bovine Serum were purchased from Sigma Aldrich. *H. pylori* strains were purchased from The American Type Culture Collection (ATCC). Tryptic Soy Agar (TSA) + 5% sheep blood (SB) plates were purchased from Fannin Ireland. Defibrinated Horse blood was purchased from Thermo Scientific. GenBags, GenBoxes and microaerophilic environment producing sachets (Biomerieux) were purchased from Cruinn Ltd.

Urease

Assays were prepared in 96-well microtiter plates. Urease enzyme (5-15 I.U.) was pre-incubated at 25 °C for 30 min with inhibitors at 0.1, 1 and 10mM in 100mM potassium phosphate buffer, pH 6.8 in 50 mL volumes. Following pre-incubation, enzyme assay mixtures were added containing 100 mM potassium phosphate buffer, pH 6.8, 150 mM urea, 0.002% phenol red in a final volume of 200 mL.[31] Linear changes in absorbance at 565 nm at 37 °C, associated with the change in absorption of phenol red due to production of ammonia, were measured over a 15 min period. Rates of inhibition were determined and plotted as percentage inhibition versus control.

Bacterial strains and culture conditions

Commercial *H. pylori* strains J99, 60190 and 26695 were routinely cultured on TSA supplemented with 5% SB. The plates were incubated at 37 °C for 4-7 days under microaerophilic conditions using GenBag Microaer™ according to the supplier's instructions.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the bismuth complexes was determined by the Agar Dilution Method, which is recommended by the Clinical

and Laboratory Standards Institute (CLSI) for susceptibility testing of *H. P*ylori.[30]

H. pylori cultures were cultured on TSA + 5% SB. Single colonies were re-suspended in 0.9 % NaCl to a density of a 4 McFarland standard (approximately 10^8 CFU mL^{-1}), measured using a Densicheck instrument (Bioemerieux Ltd). Aliquots (5 µl) of these suspensions were then dropped onto the TSA plates containing doubling dilutions of test compounds, ranging in concentration from 64 µg mL^{-1} to 0.5 µg mL^{-1}. Each compound was tested alongside BSS in comparable concentrations and a control plate with no compound as a positive control. The MIC's, defined as the lowest concentration at which no growth was observed, were determined by examination of the plates after incubation under microaerophilic conditions for 3-5 days at 37 ^oC.

Synthesis

Synthesis of $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2]$ (1)

$Bi(NO_3)_3.5H_2O$ (485 mg, 1 mmol) and benzohydroxamic acid (302 mg, 2.1 mmol) were placed in a clean dry round bottomed flask. Ethanol (50ml) was added and the mixture was stirred for 30 mins and subsequently heated to reflux for 5 hours. The clear solution was allowed to cool and the solution was filtered regardless. The clear filtrate was then condensed *in vacuo to c.* 10 ml. On the addition of H_2O (5 ml) a white solid precipitated, which was filtered, and dried to give compound **1**, 410mg (77%), ν_{max} (cm^{-1}): 1595 (s, C=O), 1560 (s, C-N), 1477, 1385 (s, N-O). Elemental analysis; ($C_{28}H_{24}Bi_2N_6O_{14}$) Calc. (Found): C 30.95 (30.84), H 2.23 (1.91), N 7.74 (7.45) %. ^1H NMR (400 MHz, CD_3OD, 25 °C): δ_H 7.85 (dd, *J* = 7.1, 1.5 Hz, 2H, Aromatic H), 7.56 (d, *J* = 7.4 Hz, 1H, aromatic H), 7.52 – 7.46 (m, 2H, Aromatic H); ^{13}C NMR (100.1 MHz, CD_3OD, 25 °C): δ_C 165.6, 133.0, 131.5, 130.1, 128.0. MS (ESI+) m/z: 271.8 ($Bi(NO_3)$), 345.7 ($Bi(Bha_{-1H})$), 407.7 ($Bi(NO_3)(Bha_{-1H})$), 480.7($Bi(Bha_{-1H})_2$). Single crystals of $[Bi_2(Bha_{-1H})_2(\mu\text{-}Bha_{-1H})_2(\eta^2\text{-}NO_3)_2].CH_3OH$ were isolated upon recrystallization of compound **1** from methanol.

Synthesis of $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)_2(OH_2)_2(Sha_{-1H})_{12}]$ (2)

$Bi(NO_3)_3.5H_2O$ (905 mg, 1.86 mmol) and salicylhydroxamic acid (856 mg, 5.59 mmol) were placed in a clean dry round bottomed flask. Ethanol (80 ml) was added and the mixture was stirred for 30 mins and subsequently heated to reflux for 5 hours. The clear solution was allowed to cool and filtered regardless. The clear filtrate was then condensed *in vacuo to c.* 10 ml. On the addition of H_2O (5 ml) a white solid precipitated, which was filtered, and dried, 750mg. ν_{max} (cm^{-1}): 1604 (s, C=O), 1560 (s, C-N), 1480, 1383 (s, N-O). Elemental analysis; 31.06, H 2.08, N 6.75 %. ^1H NMR (400 MHz, CD_3OD, 25 °C): δ_H 7.85 (d, *J* = 6.2 Hz, 1H, aromatic H), 7.31 (t, *J* = 6.9 Hz, 1H, aromatic H), 6.87 (m, 2H, aromatic H). ^{13}C NMR (100.1 MHz, CD_3OD, 25°C): δ_C 166.1, 157.6, 134.3, 130.4, 120.8, 117.2, 116.8. MS (ESI+) m/z: $[Bi(NO_3)(Sha_{-1H})]^+$ 423.9, $[Bi(Sha_{-1H})_2]^+$ 513.8.

Single crystals of $[Bi_6(CH_3OH)_2(\eta^1\text{-}NO_3)_2(\eta^2\text{-}NO_3)_2(OH_2)_2(Sha_{-1H})_{12}].(CH_3OH)_4(H_2O)_4(NO_3)_2$ **2** were isolated on recrystallization of white solid from methanol.

Crystallographic data

Complex 1

A specimen of $C_{29}H_{28}Bi_2N_6O_{15}$, approximate dimensions 0.030 mm x 0.050 mm x 0.210 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured at 100(2)K using an Oxford Cryosystems Cobra low temperature device using a MiTeGen micromount. See Table 1 for collection parameters and exposure time. Bruker APEX software was used to correct for Lorentz and polarization effects.[32]

A total of 1027 frames were collected. The total exposure time was 2.85 hours. The frames were integrated with the Bruker SAINT software package using a wide-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 32284 reflections to a maximum θ angle of 27.53° (0.77 Å resolution), of which 4213 were independent (average redundancy 7.663, completeness = 100.0%, R_{int} = 6.26%, R_{sig} = 4.44%) and 3768 (89.44%) were greater than $2\sigma(F^2)$. The final cell constants of a = 4.8467(4) Å, b = 13.1798(12) Å, c = 15.0042(14) Å, α = 74.217(3)°, β = 85.628(3)°, γ = 82.567(3)°, volume = 913.71(14) $Å^3$, are based upon the refinement of the XYZ-centroids of 2486 reflections above 20 σ(I) with 4.823° < 2θ < 43.96°. Data were corrected for absorption effects using the multi-scan method (SADABS).[33] The ratio of minimum to maximum apparent transmission was 0.620. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.4625 and 0.7456.

The structure was solved and refined using the Bruker SHELXTL Software Package,[34] using the space group Pī, with Z = 1 for the formula unit, $C_{29}H_{28}Bi_2N_6O_{15}$. The final anisotropic full-matrix least-squares refinement on F^2 with 251 variables converged at R1 = 3.71%, for the observed data and wR2 = 9.35% for all data. The goodness-of-fit was 1.084. The largest peak in the final difference electron density synthesis was 2.302 $e^-/Å^3$ and the largest hole was -1.477 $e^-/Å^3$ with an RMS deviation of 0.227 $e^-/Å^3$. On the basis of the final model, the calculated density was 2.033 g/cm^3 and F(000), 530 e^-.

Refinement Note: The phenyl ring systems were disordered 50:50% and were modelled with restraints and constraints (ISOR and EADP). The disordered MeOH solvent molecule was modelled as 50% occupied in three locations in the asymmetric unit. Restraints and constraints were applied to model the disorder (DFIX, EADP and ISOR). Each disordered MeOH moiety refined as 17:18:15% occupied.

Refinement Note: N-H and O-H hydrogen atoms were located and refined using a DFIX restraint:

O5, O6, O16, O17, O18 and N9, N20, N31, N42, N53, N64.

Complex 2

A specimen of $C_{90}H_{108}Bi_6N_{18}O_{66}$, approximate dimensions 0.050 mm x 0.080 mm x 0.150 mm, was used for the X-ray

crystallographic analysis. The X-ray intensity data were measured at 100(2)K using an Oxford Cryosystems Cobra low temperature device using a MiTeGen micromount. See Table 1 for collection parameters and exposure time. Bruker APEX software was used to correct for Lorentz and polarization effects.[32]

A total of 1731 frames were collected. The total exposure time was 4.81 hours. The integration of the data using a triclinic unit cell yielded a total of 111802 reflections to a maximum θ angle of 30.14° (0.71 Å resolution), of which 17529 were independent (average redundancy 6.378, completeness = 99.4%, R_{int} = 4.43%, R_{sig} = 3.33%) and 14300 (81.58%) were greater than $2\sigma(F^2)$. The final cell constants of a = 15.0432(6) Å, b = 15.2366(5) Å, c = 16.5434(6) Å, α = 112.5660(10)°, β = 116.5850(10)°, γ = 94.4850(10)°, volume = 2984.48(19) $Å^3$, are based upon the refinement of the XYZ-centroids of reflections above 20 σ(I). Data were corrected for absorption effects using the numerical method (SADABS).[33] The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.4747 and 0.7837.

The structure was solved and refined using the Bruker SHELXTL Software Package,[34] using the space group P$\bar{1}$, with Z = 1 for the formula unit, $C_{90}H_{108}Bi_6N_{18}O_{66}$. The final anisotropic full-matrix least-squares refinement on F^2 with 827 variables converged at R1 = 2.54%, for the observed data and wR2 = 5.33% for all data. The goodness-of-fit was 1.004. The largest peak in the final difference electron density synthesis was 1.894 $e^-/Å^3$ and the largest hole was -1.451 $e^-/Å^3$ with an RMS deviation of 0.154 $e^-/Å^3$. On the basis of the final model, the calculated density was 2.087 g/cm^3 and F(000), 1800 e^-.

Refinement Note: N-H and O-H hydrogen atoms were located and refined using a DFIX restraint:
O5, O6, O16, O17, O18 and N9, N20, N31, N42, N53, N64.

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