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# Recent advances in bioinorganic chemistry of bismuth

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Bismuth has been used in medicine for over two centuries for the treatment of various diseases, in particular for gastrointestinal disorders, owing to its antimicrobial activity. Recent structural characterization of bismuth drugs provides an insight into assembly and pharmacokinetic pathway of the drugs. Mining potential protein targets inside the pathogen via metallomic/metalloproteomic approach and further characterization on the interactions of bismuth drugs with these targets laid foundation in understanding the mechanism of action of bismuth drugs. Such studies would be beneficial in rational design of new potential drugs.

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## Introduction

Bismuth compounds have been used in medicine for more than 200 years for the treatment of various diseases including syphilis, hypertension, infections, skin conditions, and gastrointestinal disorders [1,2•]. The discovery of *Helicobacter pylori* (*H. pylori*), a Gram-negative bacterium from gastric mucosa that is responsible for gastric and duodenal ulcers [3], has further promoted both research and medical applications of bismuth. Currently, three bismuth drugs, that is, bismuth subsalicylate (Pepto-Bismol®), colloidal bismuth subcitrate (De-Nol®), and ranitidine bismuth citrate (Tritec® and Pylorid®) are being used worldwide in combination with antibiotics to eradicate *H. pylori* infection. In addition, many new bismuth compounds with different structures and activities as well as bismuth nanotubes have been synthesized [2•,4,5].

For the past few years, enormous efforts have been made towards understanding the structures [6,7••,8] as well as the mechanism of actions of the bismuth drugs [2•]. Recent advances in biophysics and molecular biology have provided invaluable tools necessary to study the bioinorganic chemistry of bismuth. For example, time-resolved inductively coupled plasma mass spectrometry enables bismuth antiulcer drugs to be tracked in single *Helicobacter pylori* cells (ca. $1.0 \times 10^6$ Bi atoms/cell). The uptake of bismuth by *Helicobacter pylori* is retarded by ferric ions ($Fe^{3+}$) suggesting that bismuth drugs may utilize certain iron-transport pathways in the pathogen [9]. A number of protein targets of bismuth drugs, in particular those from the *H. pylori* have been identified by metallomic and metalloproteomic approach [10•,11]. Several comprehensive reviews summarized the chemistry and biological chemistry of bismuth as well as its medical applications are available recently [1,2•,6,11,12]. Here we will only focus on some recent advances in the structures and protein targets of bismuth drugs as well as design of potentially active new bismuth complexes.

## Structural models of bismuth drugs

In spite of extensive clinical usage of bismuth drugs, the structures of these drugs have not been unveiled until the past two decades. Various bismuth citrate complexes with different bismuth to citrate ratios have been crystallized at different pH values. Among these, the structure obtained under acidic condition (pH 3) most probably represents real situation in the stomach [13]. Colloidal bismuth subcitrate (CBS) is likely to assemble into sheets and then three-dimensional polymers using bismuth citrate dinuclear units $[Bi(cit)_2Bi]^{2-}$ as building blocks to form a protective coating on the ulcer craters [13].

Crystal structures of four bismuth citrate complexes obtained at the acidic pH values in the presence of either ethylenediamine or pyridine [14] reveal that bismuth citrate dimeric units $[Bi(cit)_2Bi]^{2-}$ (Figure 1a) serve as the basic building blocks leading to polymeric structures with regular meshes and internal cavities. The protonated ethylenediamine, despite unobservable in the structure (Figure 1b), and pyridine moieties (Figure 1c) are embedded probably by diffusion or electrostatic interaction in the polymeric framework to achieve charge balance since all citrate anions found in the structures are deprotonated leading to citrate tetrannions ($[C_6H_4O_7]^{4-}$). The composition of the bismuth citrate complex frameworks depends on the size and level of protonation of the inserted cations, the pH values as well as the time of crystallization. The structural model of ranitidine bismuth citrate (RBC) was constructed based on the framework of crystal structure of complex 1 [14]. Ranitidine molecules can be readily embedded into the cavities perpendicular to the *bc* plane with H-bonds formed between ranitidine and the bound citrate ligands,

Figure 1d. In addition, the sulfur atoms of ranitidine form H-bonds with a water molecule that coordinates to bismuth. Based on X-ray crystal structures as well as ESI-MS of bismuth citrate complexes, it is likely that bismuth citrate-based drugs degrade under gastric acidic condition (pH $\sim$ 3), from 3-D polymer framework to 2-D polymeric sheets and finally to a basic dimeric unit such as $[Bi(cit)_2Bi]^{2-}$ that can be absorbed or transported by membrane receptors.

Bismuth subsalicylate (Pepto-Bismol®, BSS) is one of the most commonly used bismuth drugs [11]. Structural characterization of bismuth carboxylates is challenging owing to difficulties in controlling hydrolysis of these complexes or the formation of coordination oligomers/polymers. Recently, structures $[Bi_{38}O_{44}(Hsal)_{26}(Me_2CO)_{16}(H_2O_2)]\cdot(Me_2CO_4)$ and $[Bi_9O_7(Hsal)_{13}(Me_2CO)_5]\cdot(Me_2CO)_{1.5}$ (Figure 1e) may provide an insight into the nature of BSS and a basis for studies the mode of action of the drug [8]. The crystals of $[Bi_9O_7(Hsal)_{13}(Me_2CO)_5]\cdot(Me_2CO)_{1.5}$ initially predominate with only relative small amounts of the large cluster with 38 bismuth atoms that appeared to be the least soluble and most thermodynamically stable form when extending crystal growth times. The structure contains a basic building block $[Bi_6O_8]^{2+}$ polyhedron as found in other bismuth oxo clusters [15], which have six octahedral Bi atoms with the eight O atoms located over all of the triangular faces. The cores of the two structures are the $Bi_9$ clusters comprised of a central $Bi_6$ octahedron but with only seven of the eight trigonal faces capped by an O atom as shown in Figure 1f. In both structures, bismuth also coordinated to the solvent molecules of acetone, which can be replaced by other solvent molecules such as DMF (dimethyflormamide) when crystallized in this solvent [7••]. Interestingly, the core of $[Bi_9O_7(Hsal)_{13}(Me_2CO)_5]\cdot(Me_2CO)_{1.5}$ lies at the heart of $[Bi_{38}O_{44}(Hsal)_{26}(Me_2CO)_{16}(H_2O_2)]\cdot(Me_2CO_4)$ combined with the presence of Bi-coordinated solvent molecules in these structures reveals a possible process for hydrolysis and core formation, and indicates that the complex $[Bi_9O_7(Hsal)_{13}(Me_2CO)_5]\cdot(Me_2CO)_{1.5}$ gave rise to the complex $[Bi_{38}O_{44}(Hsal)_{26}(Me_2CO)_{16}(H_2O_2)]\cdot(Me_2CO_4)$.

The first structure of BSS without organic auxiliaries obtained from X-ray diffraction data of bismuth disalicylate powder [7••] gave rise to two-dimensional polymers held by Bi-O linkages and O–H$\cdots$O hydrogen bonds with one bismuth atom, one salicylate mono-anion and one salicylate dianion in each unit. The monoanionic salicylate coordinates to a single $Bi^{3+}$ ion through its carboxylate group only and the dianionic salicylate employs the phenoxide oxygen atoms as bridging ligands to form four-membered $Bi_2O_2$ rings. An additional oxygen atom from a water molecule is bonded to bismuth. Such a structure of BSS resembles bismuth complexes with substituted benzoic acids [16].

## New bismuth complexes and their activities

Over the past decade, significant work has been devoted into the development of new bismuth drugs [2•,17,18]. Various new bismuth-containing complexes have been synthesized and showed promising *in vitro* activities against *H. pylori* [17,18]. Importantly, some bismuth-containing complexes have been demonstrated to exhibit new *in vitro* activities including antifungal, antiviral or even anticancer activities [19–24].

Bismuth drugs such as BSS, CBS and RBC are effective in treating and eradicating *Helicobacter pylori* together with antibiotics. However, these non-steroidal anti-inflammatory drugs (NSAIDs) may also cause gastrointestinal damage. Bismuth derivatives of NSAIDs exhibited good *in vitro* activity against the three strains of *H. pylori* with the minimum inhibitory concentrations (MIC) $\geq$ 6.25 μg/mL, which are better than commercially used BSS (8 μg/mL), laboratory prepared bismuth salicylate ($\geq$12.5 μg/mL) and CBS ($\geq$12.5 μg/mL) [18]. Therefore, these compounds may have great potential in the treatment of *H. pylori* infection while allowing the concomitant therapeutic benefits of NSAID treatment. Similarly, heteroleptic bismuth sulfosalicylate complexes $[PhBi(HSsal)H_2O]_\propto$ and $[PhBi(HSsal)H_2O]_\propto$ as well as *bis*-phenylbismuth sulfonates $[Ph_2Bi(O_3SR)]_\propto$ (R = *p*-toly, mesityl or S-(+)-10-camphoryl) [17,25] showed significant activities against *H. pylori* with MIC < 6.25 μg/mL. Some cyclic organobismuth compounds bearing a nitrogen or sulfur atoms as additional ring member also exhibit various antibacterial activities including Gram-positive and Gram-negative bacteria [26]. Although these bismuth-containing complexes showed *in vitro* activities against *H. pylori* and other pathogens, there appears to be lack of *in vivo* data and more works are warranted to promote medical application of these complexes.

Beside antibacterial activity, bismuth complexes of substituted benzoic acids also exhibit significant anti-Leishmanial activity against the promastigotes of L. major V121 [27]. Heterocyclic organobismuth compounds [ClBi(5-R-$C_6H_3$-2-$SO_2C_6H_4$-1′-) (R = Me, Ph, MeO, Cl, H, t-Bu, $CF_3$, F, $Me_2N$) exert antifungal activities against *Saccharomyces cerevisiae* and the activity depends on the lipophilicity of the compounds: the higher the lipophilicity, the lower the antifungal activity [19]. Some organobismuth compounds have been also found to exhibit anti-tumor potentials [2•,24]. Bismuth xanthate complexes $[Bi(S_2COR)_3]$ (R = Et, i-Pr, cyclohexyl) were shown to exert cytotoxic activities against Calu-6 (lung adenocarcinoma) with a similar $IC_{50}$ values of cisplatin [28], indicating that these compounds have potency comparable to cisplatin. Moreover, these bismuth complexes also exerted cytotoxic activities against cisplatin-insensitive MCF-7 (mammary carcinoma) [28]. Bismuth dithiocarbamate complexes with general formula of $[Bi(S_2CNR_2)]$ were also demonstrated to exhibit potent