Dinuclear copper(I) complexes of N-methylbenzothiazole-2-thione: synthesis, structures, antibacterial activity and DNA interaction

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ABSTRACT
Three copper(I) halide complexes containing N-methylbenzothiazole-2-thione (mbtt) and triphenylphosphine (PPh3) have been prepared and structurally characterized by X-ray single-crystal analysis. Copper(I) halide precursors [CuΧ(PPh3)]4 (Χ = Cl, Br, I) react with mbtt in 1 : 4 M ratio to give complexes of formula [CuΧ(mbtt)(PPh3)]2. Hereby, dimerization is achieved in case of copper(I) chloride and bromide via halide bridges, while copper(I) iodide gives the binuclear thione-S-bridged dimer. The new complexes show moderate in vitro antibacterial activity against certain bacterial strains. The interaction of the compounds with calf-thymus DNA was monitored via UV–vis spectroscopy, DNA-viscosity measurements and their competition with ethidium bromide for the DNA intercalation sites studied by fluorescence emission spectroscopy. Intercalation was revealed as the probable mode of binding.

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1. Introduction

In the past three decades, heterocyclic thiones attracted attention as soft Lewis base donors in complexes of closed-shell $d^{10}$ metal ions for use in bioinorganic chemistry due to the close relevance of these molecules to biological systems [1, 2]. In this regard, in vitro pharmacological activities, such as antiviral, antibacterial or antifungal, were confirmed for a number of imidazole-, thiazole-, thiadiazole-, oxazole-, uracil-, and hydantoin-based derivatives as well as for some of their copper(I) and silver(I) complexes [3–8].

The role of copper in fundamental biochemical processes is well-known. Its desired redox behavior, i.e. the easy interchange between its two prevailing oxidation states, but also its affinity for oxygen and for functional groups of proteins, make copper a useful co-factor for numerous enzymes involved in biological functions like electron transfer, oxygen transport, and other processes in cells. Current biochemistry-oriented research on copper complexes increasingly focus on their potential use as antibacterial [9, 10], antitumor [11–13], or anti-inflammatory [14, 15] agents. Most compounds tested are copper(II) complexes, while similar studies on copper(I) are less common.

In a recent study on copper(I) halide complexes of N-methylbenzothiazole-2-thione (hereafter abbreviated as mbtt), we noticed that triphenylphosphine (PPh$_3$) containing mononuclear species [CuX(PPh$_3$)$_2$(mbtt)] showed significant activity against several Gram-positive and Gram-negative bacteria while their phosphine-free dinuclear counterparts [CuX(mbtt)$_2$]$_2$ were practically inactive [16]. Further, a similarly high in vitro antibacterial activity was also found for the analogous mononuclear complexes [CuX(xantphos)(mbtt)] [xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethyl-xanthene] formed by a chelating diphosphine [17], as well as for the phosphine-free silver(I) complexes [(mbtt)$_2$Ag($\mu$-mbtt)$_2$Ag(mbtt)$_2$]$^\mathrm{2+}$ and [Ag(mbtt)$_2$]$^\mathrm{2+}$[CF$_3$SO$_3$] [18]. To obtain more information on the above interesting findings and to further contribute to clarification of the relationship between structure and bioactivity in such thione/phosphine mixed ligand copper(I) and silver(I) complexes, we now report the synthesis, characterization, and antibacterial activity of three new dinuclear copper(I) halide complexes containing the same heterocyclic thioamide. Relatively little attention has been paid to coordination chemistry of mbtt so far, including complexes of antimony(III), tellurium(IV), and gold(I) [19–22]. Further, we investigate the interaction of the compounds (i.e. mbtt and its complexes 1–3) with calf-thymus (CT) DNA directly by UV–vis spectroscopy and viscosity measurements and indirectly via the ethidium bromide (EB) displacement ability of the complexes from the EB–DNA conjugate by fluorescence emission spectroscopy, in order to determine the interaction mode and to calculate the DNA-binding constants of the complexes.

2. Experimental

2.1. Materials and instrumentation

Commercially available copper(I) halides, triphenylphosphine, N-methylbenzothiazole-2-thione, CT DNA, EB, NaCl and trisodium citrate were purchased from Sigma–Aldrich Co. and all solvents were purchased from Chemlab. All chemicals and solvents were of reagent grade and were used as purchased without purification. Precursors of type [CuX(PPh$_3$)$_2$]$_2$ were prepared according to a literature procedure [23].

DNA stock solution was prepared by dilution of CT DNA to buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0) followed by exhaustive stirring for three days, and kept at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm ($A_{260}/A_{280}$) of $\sim$1.90, indicating that the DNA was sufficiently free of protein contamination [24]. The DNA concentration was determined by the UV absorbance at 260 nm after 1 : 20 dilution using $\varepsilon$ = 6600 M$^{-1}$ cm$^{-1}$ [25].

Infrared spectra from 4000 to 200 cm$^{-1}$ were recorded in KBr disks with a Nicolet FT-IR 6700 spectrophotometer. UV–visible (UV–vis) spectra were recorded as Nujol mulls and in DMSO solution at concentrations from $10^{-5}$ to $10^{-3}$ M on a Hitachi U-2001 dual beam spectrophotometer. The $^1$H-Nuclear...
Magnetic Resonance (NMR) spectra were recorded at 300 MHz on a Bruker AM 300 spectrometer in CDCl₃ using tetramethylsilane as an internal standard. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18 mL LCP spindle.

2.2. Crystal structure determination

Single-crystals of 1–3 suitable for crystal structure analysis were obtained by slow evaporation of their mother liquids at room temperature. For the structure determination, single-crystals of the compounds were mounted on a Bruker Kappa APEX II diffractometer equipped with a triumph monochromator. Data were corrected for absorption effects using the multi-scan method (SADABS) [26]. The collected frames were integrated with the Bruker SAINT software package [27] using a narrow-frame algorithm. The structures were solved using SUPERFLIP package [28] and refined by full-matrix least-squares method on $F^2$ using the CRYSTALS package version 14.00 [29]. All non-hydrogen, non-disordered atoms were refined anisotropically. All hydrogens were found at expected positions and refined using soft constraints. By the end of the refinement, they were positioned using riding constraints. The crystal data and some details of the data collection and structure refinement for 1–3 are given in table 1. Illustrations were generated with CAMeRoN [30].

2.3. General procedure for the synthesis of 1–3

To a solution of 0.125 mmol of [CuX(PPh₃)]₄ (181 mg for X = Cl, 203 mg for X = Br and 226 mg for X = I) in 50 mL of dry acetonitrile was treated with N-methylbenzothiazole-2-thione (90.5 mg, 0.5 mmol) dissolved in a small amount (~20 mL) of dry methanol and the reaction mixture was stirred for 2 h at 50 °C. Slow evaporation of the resulting yellow solution at ambient conditions afforded a microcrystalline solid, which was filtered off and dried in vacuo.

| Compound | 1 | 2 | 3 |
|----------|---|---|---|
| Formula  | C₅₂H₄₄Cu₂Cl₂N₂P₂S₄ | C₅₂H₄₄Cu₂Br₂N₂P₂S₄ | C₅₂H₄₄Cu₂I₂N₂P₂S₄ |
| Formula weight | 1085.14 | 1174.05 | 1242.17 |
| Crystal system | Monoclinic | Monoclinic | Triclinic |
| Temperature | 293 K | 293 K | 293 K |
| Wavelength | 0.71073 Å | 0.71073 Å | 0.71073 Å |
| Space group | C2/c | C2/c | P1 |
| Unit cell dimensions | a = 27.8423(5) Å | a = 27.5519(14) Å | a = 10.2070(3) Å |
| | b = 10.0659(2) Å | b = 10.2556(6) Å | b = 10.8561(3) Å |
| | c = 18.9930(4) Å | c = 19.2087(12) Å | c = 13.4958(4) Å |
| | α = 90° | α = 90° | α = 100.646(2°) |
| | β = 113.5550(10)° | β = 114.225(2)° | β = 107.124(2)° |
| | γ = 90° | γ = 90° | γ = 112.581(2)° |
| Z | 4 | 4 | 4 |
| Absorption coefficient (μ) | 1.257 mm⁻¹ | 2.745 mm⁻¹ | 2.369 mm⁻¹ |
| Density (Calcd) | 1.48 g cm⁻³ | 1.58 Mg m⁻³ | 1.69502 g cm⁻³ |
| θ range for data collection | 1.596 to 33.855 | 1.621 to 26.658 | 1.677 to 33.761 |
| F (0 0 0) | 2224 | 2368 | 628 |
| Reflections collected | 52,292 | 38,913 | 50,761 |
| Independent reflections | 9805 | 5036 | 9774 |
| Completeness up to θ | 99.9% (θ = 30.118) | 98.4% (θ = 26.658) | 99.9% (θ = 28.298) |
| Data/restraints/parameters | 4854/0/289 | 2930/0/289 | 4853/0/289 |
| Goodness-of-fit on F² | 0.999 | 0.999 | 1.000 |
| Final R indices [I > 2σ(I)] | R₁ = 0.033 | R₁ = 0.064 | R₁ = 0.061 |
| | wR₂ = 0.067 | wR₂ = 0.090 | wR₂ = 0.091 |
| Largest diff. peak and hole | 0.60, −0.37 e Å⁻³ | 1.20, −1.20 e Å⁻³ | 2.22, −1.34 e Å⁻³ |
2.3.1. \([\text{CuCl}(\text{PPh}_3)(\text{mbtt})]_2\) (1)

Yellow crystals (30.5 mg, 45%), m.p. 164 °C; Anal. Calcd for C\(_{52}\)H\(_{44}\)Cu\(_2\)Cl\(_2\)N\(_2\)P\(_2\)S\(_4\): C, 57.56; H, 4.09; N, 2.58. Found: C, 57.47; H, 3.92; N, 2.58. IR (cm\(^{-1}\)): 3077m, 3054m, 2929w, 1587m, 1461vs, 1420vs, 1351vs, 1318vs, 1162s, 1138vs, 1097vs, 982vs, 820s, 761vs, 536vs, 509vs, 432m; UV–vis (λ\(_{\text{max}}\), log ε): 227 (4.27), 238 (4.09), 258 (4.56); 1H NMR (CDCl\(_3\), 300 MHz) δ: 3.90 (s, 3H, N-CH\(_3\)), 7.30–7.35 (m, 6H, Ph), 7.35–7.41 (m, 3H, Ph), 7.44–7.47 (m, 1H, mbtt), 7.49–7.55 (m, 7H, Ph + mbtt), 7.61–7.70 (m, 2H, mbtt).

2.3.2. \([\text{CuBr}(\text{PPh}_3)(\text{mbtt})]_2\) (2)

Yellow crystals (46 mg, 63%), m.p. 171 °C; Anal. Calcd for C\(_{52}\)H\(_{44}\)Cu\(_2\)Br\(_2\)N\(_2\)P\(_2\)S\(_4\): C, 53.20; H, 3.78; N, 2.38. Found: C, 53.27; H, 3.83; N, 2.28. IR (cm\(^{-1}\)): 3066m, 3048m, 2925w, 1583m, 1476s, 1434vs, 1347vs, 1314vs, 1269s, 1142vs, 1094vs, 978vs, 819s, 747vs, 694vs, 516vs, 507vs, 428m; UV–vis (λ\(_{\text{max}}\), log ε): 227 (4.27), 238 (4.09), 258 (4.56); UV–vis (λ\(_{\text{max}}\), log ε): 226sh (4.00), 238 (3.82), 259 (3.67), 323 (3.74); 1H NMR (CDCl\(_3\), 300 MHz) δ: 3.87 (s, 3H, N-CH\(_3\)), 7.28–7.33 (m, 6H, Ph), 7.34–7.39 (m, 3H, Ph), 7.44–7.47 (m, 1H, mbtt), 7.49–7.55 (m, 7H, Ph + mbtt), 7.64–7.70 (m, 2H, mbtt).

2.3.3. \([\text{CuI}(\text{PPh}_3)(\text{mbtt})]_2\) (3)

Yellow crystals (52 mg, 53%), m.p. 118 °C; Anal. Calcd for C\(_{52}\)H\(_{44}\)Cu\(_2\)I\(_2\)N\(_2\)P\(_2\)S\(_4\): C, 49.25; H, 3.50; N, 2.21. Found: C, 49.55; H, 3.55; N, 2.31. IR (cm\(^{-1}\)): 3063w, 2925w, 1585m, 1460vs, 1433vs, 1413s, 1344vs, 1312vs, 1261s, 1141vs, 1096vs, 1065s, 966vs, 821s, 748vs, 717s, 534s, 521s, 510s, 425m; UV–vis (λ\(_{\text{max}}\), log ε): 227 (4.27), 238 (4.09), 258 (4.56); UV–vis (λ\(_{\text{max}}\), log ε): 226sh (4.00), 238 (3.82), 259 (3.67), 323 (3.74); 1H NMR (CDCl\(_3\), 300 MHz) δ: 3.87 (s, 3H, N-CH\(_3\)), 7.30–7.33 (m, 6H, Ph), 7.34–7.39 (m, 3H, Ph), 7.42–7.45 (m, 3H, Ph), 7.49–7.52 (m, 7H, Ph + mbtt), 7.58–7.61 (m, 1H, mbtt), 7.67–7.74 (m, 2H, mbtt).

2.4. Materials and methods for antimicrobial tests

The antibacterial activities of 1–3 against four bacterial species \([\text{Escherichia coli (XL1)} (\text{E. coli})], \text{Staphylococcus aureus (NCIM 2079}) (\text{S. aureus}), \text{Bacillus subtilis (ATCC 6633}) (\text{B. subtilis}), \text{and Bacillus cereus (ATCC11778}) (\text{B. cereus})\] were estimated by the minimum inhibitory concentration (MIC) method as described earlier [14]. Two different cultivation media were used for antimicrobial activity tests: (i) the Luria-Bertani broth containing 1% (w/v) tryptone, 0.5% (w/v) NaCl, and 0.5% (w/v) yeast extract was used and (ii) the minimal medium salts broth containing 1.5% (w/v) glucose, 0.5% (w/v) NH\(_4\)Cl, 0.5% (w/v) K\(_2\)HPO\(_4\), 0.1% (w/v) NaCl, 0.01% (w/v) MgSO\(_4\)-7H\(_2\)O, and 0.1% (w/v) yeast extract. The pH of the media was adjusted to 7.0.

2.5. Binding studies with CT DNA

In order to study the binding properties of mbtt and 1–3 with CT DNA, the compounds were initially dissolved in DMSO (1 mM) due to low solubility of the compounds in H\(_2\)O. Mixing of such solutions with the aqueous buffer DNA solutions used in the studies never exceeded 5% DMSO (v/v) in the final solution. All studies were performed at room temperature. Control experiments with DMSO were performed and no changes in the spectra of the CT DNA were observed. The interaction of the compounds with CT DNA was studied by UV spectroscopy and viscosity measurements; furthermore, the ability of the compounds to displace EB from the EB–DNA conjugate was examined by fluorescence emission spectroscopy.

2.5.1. DNA-binding studied by UV spectroscopy

The interactions of mbtt and 1–3 with CT DNA were studied by UV spectroscopy in order to investigate their binding to CT DNA and to calculate the corresponding DNA-binding constants (K\(_b\)). The UV spectra of CT DNA were recorded for a constant DNA concentration in the presence of each compound at diverse [complex]/[DNA] mixing ratios (= r). Additionally, the UV–vis spectra of the compounds were
recorded for a constant concentration of CT DNA at diverse [DNA]/[complex] mixing ratios (\( r \)). The DNA-binding constant (\( K_b \) in M\(^{-1}\)) was determined based on the changes in the absorbance at the corresponding \( \lambda_{\text{max}} \) with increasing concentrations of CT DNA and it is calculated by the ratio of slope to the y intercept in plots [DNA]/(\( \epsilon_A - \epsilon_I \)) versus [DNA], according to the Wolfe–Shimer equation [31]:

\[
\frac{[\text{DNA}]}{(\epsilon_A - \epsilon_I)} = \frac{[\text{DNA}]}{(\epsilon_b - \epsilon_I)} + \frac{1}{K_b (\epsilon_b - \epsilon_I)}
\]

where [DNA] is the concentration of DNA in base pairs, \( \epsilon_A = \frac{A_{\text{obsd}}}{[\text{compound}]}, \) \( \epsilon_I = \) the extinction coefficient for the free compound, and \( \epsilon_b = \) the extinction coefficient for the compound in the fully bound form.

### 2.5.2. DNA-viscosity measurements

The viscosity of a DNA solution ([DNA] = 0.1 mM in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0)) was measured in the presence of increasing amounts of mbtt and complexes 1–3 (up to the \( r \) value = 0.34). All measurements were performed at room temperature. The obtained data are presented as \((\eta/\eta_0)^{1/3} \) versus \( r \), where \( \eta \) is the viscosity of DNA in the presence of the compound and \( \eta_0 \) is the viscosity of DNA alone in buffer solution.

### 2.5.3. EB-competition studied by fluorescence emission spectroscopy

The competition of the compounds (i.e. mbtt and 1–3) with EB (the ability of the complexes to displace EB from its DNA–EB conjugate and its extent) was studied by fluorescence emission spectroscopy. The DNA–EB conjugate was prepared by pretreating 20 \( \mu \)M EB and 26 \( \mu \)M CT DNA in buffer (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) for 1 h. The possible intercalating effect of the compounds was studied by adding stepwise a certain amount of a solution of the compound into the DNA–EB conjugate solution. The influence of the addition of each compound to the DNA–EB conjugate was obtained by monitoring the changes in fluorescence emission spectra of EB–DNA system with excitation wavelength \( (\lambda_{\text{ex}}) \) at 540 nm [32]. The compounds did not show any fluorescence emission bands at room temperature in solution or in the presence of DNA or EB under the same experimental conditions (\( \lambda_{\text{ex}} = 540 \) nm); therefore, the observed quenching may be attributed to displacement of EB from its EB-DNA conjugate. The Stern–Volmer constant (\( K_{SV} \) in M\(^{-1}\)) is used to evaluate the quenching efficiency for each compound according to the Stern–Volmer equation (equation 2) [32],

\[
\frac{I_0}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} [Q]
\]

where \( I_0 \) and \( I \) are the EB–DNA fluorescence emission intensities in the absence and presence of the quencher, respectively, \([Q]\) is the concentration of the quencher (i.e. mbtt and 1–3); \( K_{SV} \) is determined from the Stern–Volmer plots by the slope of the diagram \( I_0/I \ versus [Q] \). Taking \( \tau_0 = 23 \) ns as the fluorescence lifetime of the EB–DNA system [33], the corresponding quenching constants (\( k_q \), M\(^{-1}\) s\(^{-1}\)) were calculated according to equation 3.

\[
K_{SV} = k_q \tau_0
\]

### 3. Results and discussion

#### 3.1. Preparative considerations

In the majority of their copper(II) complexes, heterocyclic thiones are found to preferably coordinate through the exocyclic thione sulfur, forming either mononuclear or thione-S bridged dinuclear species involving the metal in a tetrahedral environment [1(b)]. In the specific case of mixed-ligand copper(II) halide complexes containing tertiary arylphosphines as a second bulky \( \sigma\)-donor/\( \pi\)-acceptor ligand, formation of symmetrical dicopper(II) species containing the exocyclic sulfur in a \( \mu_2\)-S bridging mode...
or the halides as bridging ligands in a Cu₂(μ-X)₂ core structure [35] is particularly widespread. Earlier observations in such complexes showed the halide bridges to be preferably formed by the “soft” iodide rather than the “hard” chloride, which led us to conclude that the kind of bridges being formed depends on the nature of the halide present. Meanwhile, several examples of related complexes were reported, in which just as in the present case, a reverse trend can be noticed, so we need to revise our view.

Compounds 1–3 have been prepared according to the reaction shown in scheme 1 using precursors [CuX(PPh₃)]₄, which proved to be the best choice for the production of dinuclear complexes. According to the elemental analyses, the crystalline materials obtained by slow evaporation of the respective CH₃CN/CH₃OH mother liquids corresponded to the formula [CuX(PPh₃)(mbtt)]. Single-crystal X-ray diffraction analysis revealed the dimeric structure of the complexes, which can be formulated as (mbtt)(PPh₃)Cu(μ-Cl)₂Cu(mbtt)(PPh₃) (1), (mbtt)(PPh₃)Cu(μ-Br)₂Cu(mbtt)(PPh₃) (2), and (PPh₃)ICu(μ-S-mbtt)Cu(PPh₃) (3) (scheme 1). Solutions of the diamagnetic complexes in acetonitrile and DMSO are non-conducting and air stable when stored in darkness at room temperature.

### 3.2. Spectroscopy

The electronic absorption spectra of the complexes, recorded in acetonitrile at room temperature, show four intense bands with maxima at ~227, ~238, ~258, and ~323 nm. With reference to the absorption spectrum of the uncoordinated mbtt, the high energy bands at 227 and 238 nm can be attributed to intraligand π → π* transitions on the thione ligand. These bands are of increased intensity relatively to those observed in the spectrum of free mbtt. The lower energy band can be considered as an almost pure charge-transfer transition at the C=S bond [36] since it remains practically unshifted upon coordination of mbtt to copper. Further, the band at 258 nm can be considered as a phosphine originating intraligand band.

The infrared spectra of 1–3, recorded from 4000 to 200 cm⁻¹, are dominated by characteristic bands due to the presence of N-methylbenzothiazole-2-thione. In particular, the intense bands at 1412 and 1260 cm⁻¹ in the spectrum of free mbtt, attributed to the C–N stretching vibration of the thioamide moiety, appear in spectra of the complexes blue-shifted (by ca. 8–20 cm⁻¹), indicating an exclusive S-coordination of the ligand. On the other hand, the expected red-shift due to S-coordination for the band at 1095 cm⁻¹ assigned to C=S stretch [37] is very small or even zero, consequently the infrared spectra of the complexes do not provide valuable information with regard to the ligand’s coordination mode.

Scheme 1. Formation of dicopper(I) complexes 1–3.
3.3. Description of the structures

The X-ray crystal structures of \([\text{CuCl}(\text{PPh}_3)(\text{mbtt})]_2\) (1), \([\text{CuBr}(\text{PPh}_3)(\text{mbtt})]_2\) (2), and \([\text{CuI}(\text{PPh}_3)(\text{mbtt})]_2\) (3) (details of crystal and structure refinement are shown in Table 1) have been determined. Compounds 1 and 2 crystallize in the monoclinic system \(\text{C}_2/c\), each with four discrete molecules in the unit cell, while 3 crystallizes in the triclinic system \(\text{P}\overline{1}\) with four discrete molecules in the unit cell. Tables 2–4 list selected bond distances and angles of these complexes, and perspective drawings with atom numbering are shown in Figures 1–3.

The structures of 1 and 2 are similar having common the planar \(\text{Cu}_2(\mu-X)_2\) core in which the distorted tetrahedral environment of each Cu ion is completed by the P of a phosphine unit and the exocyclic S of a neutral mbtt molecule.

Within the planar \(\text{Cu}_2X_2\) central core of 1 and 2, the \(\mu-\text{Cl}\) or \(\mu-\text{Br}\) bridge the two metal centers in an asymmetric manner [Cu(1)—Cl(1) = 2.5949(5) Å, Cu(1)—Cl(1)# = 2.7787(6) Å, and Cu(1)—Br(1) = 2.4374(8) Å, Cu(1)—Br(1)# = 2.5830(9) Å]. This remarkable asymmetry in the two bridging Cu—X bond distances has also been observed before in similar complexes [28, 29]. There are marked angular distortions from the ideal tetrahedral geometry around each metal center, with angles ranging from 99.20(2)° to 120.16(2)° in 1 and 102.04(3)° to 120.37(5)° in 2, whereby in both cases the largest angle surprisingly does not arise between the two bulkier ligands. The Cu—S and Cu—P distances fall in the range of values found for other copper(I) complexes with terminal thione-S and triarylphosphine ligation. The Cu—Cu separations of 3.0943(6) Å in 1 and 3.1601(13) Å in 2 are too long to represent metal-metal interaction [38].

Unlike 1 and 2, the structure of 3 features a centrosymmetric dimer in which the two Cu ions are doubly bridged by the exocyclic sulfur of two mbtt units to form a strictly planar \(\text{Cu}_2S_2\) core with a

**Table 2.** Selected bond distances (Å) and angles (°) in \([\text{CuCl}(\text{PPh}_3)(\text{mbtt})]_2\) (1).

| Bond          | Distance (Å) | Bond angle (°) |
|---------------|--------------|----------------|
| Cu(1)—Cl(1)  | 2.3226(6)    | Cl(1)—Cu(1)—Cl(1)# 99.20(2) |
| Cu(1)—Cl(1)# | 2.4492(6)    | Cu(1)—Cl(1)—Cu(1)# 80.80(2)  |
| Cu(1)—S(1)   | 2.3408(7)    | Cl(1)—Cu(1)—S(1) 120.16(2)  |
| Cu(1)—P(1)   | 2.2296(6)    | Cl(1)#—Cu(1)—S(1) 105.15(3)  |
| S(1)—C(1)    | 1.666(2)     | Cl(1)—Cu(1)—P(1) 117.36(2)  |
| Cu(1)···Cu(1)# | 3.0943(6)   | P(1)—Cu(1)—S(1) 102.83(3)  |

**Table 3.** Selected bond distances (Å) and angles (°) in \([\text{CuBr}(\text{PPh}_3)(\text{mbtt})]_2\) (2).

| Bond          | Distance (Å) | Bond angle (°) |
|---------------|--------------|----------------|
| Cu(1)—Br(1)  | 2.4374(8)    | Br(1)—Cu(1)—Br(1)# 102.04(3) |
| Cu(1)—Br(1)# | 2.5830(9)    | Cu(1)—Br(1)—Cu(1)# 80.80(2)  |
| Cu(1)—S(1)   | 2.3215(16)   | Br(1)—Cu(1)—S(1) 120.37(5)  |
| Cu(1)—P(1)   | 2.2415(14)   | Br(1)#—Cu(1)—S(1) 103.91(5)  |
| S(1)—C(1)    | 1.682(6)     | Br(1)—Cu(1)—P(1) 114.80(4)  |
| Cu(1)···Cu(1)# | 3.1601(13)  | P(1)—Cu(1)—S(1) 105.32(6)  |

**Table 4.** Selected bond distances (Å) and angles (°) in \([\text{CuI}(\text{PPh}_3)(\text{mbtt})]_2\) (3).

| Bond          | Distance (Å) | Bond angle (°) |
|---------------|--------------|----------------|
| Cu(1)—S(1)   | 2.3203(13)   | S(1)—Cu(1)—S(1)# 87.42(5)   |
| Cu(1)—S(1)#  | 2.5763(15)   | Cu(1)—S(1)—Cu(1)# 92.58(5)  |
| Cu(1)—I(1)   | 2.5400(7)    | I(1)—Cu(1)—S(1) 126.58(4)  |
| Cu(1)—P(1)   | 2.2442(13)   | S(1)#—Cu(1)—P(1) 104.74(4)  |
| S(1)—C(1)    | 1.675(5)     | I(1)—Cu(1)—P(1) 113.66(4)  |
| Cu(1)···Cu(1)# | 3.5439(13)  | P(1)—Cu(1)—I(1) 113.78(5)  |

...
Cu⋯Cu separation of 3.5439(13) Å, which is much larger than twice the van der Waals radius of copper, excluding the possibility of intermetallic bonding interactions. The distorted tetrahedral coordination around each Cu ion is completed by the P of a phosphine and a terminally bonded iodide. Interatomic bond distances and angles involving the central metal are comparable to those found in related complexes, but the marked feature of the present structure is the large asymmetry in the two bridging Cu–S bond distances of 2.3203(13) and 2.5763(15) Å, which represent the largest asymmetry observed so far in related structures.

As already mentioned, dimerization in the cuprous halide complexes bearing heterocyclic thiones and triarylphosphine ligands is common and many doubly bridged dimers, constructed on the basis of an asymmetric Cu$_2$(μ-S)$_2$ or Cu$_2$(μ-X)$_2$ core, respectively, have been observed so far. According to the
structures presented herein, the participation (or not) of the halide in the bridges preferably formed in each case does not seem to be exclusively related to its softness (or hardness).

### 3.4. Antibacterial activity

The antimicrobial activities of N-methylbenzo-thiazole-2-thione, triphenylphosphine and the copper(I) halide complexes 1–3 (average of three measurements) were estimated by monitoring the growth of certain Gram-positive (B. subtilis, B. cereus, S. aureus) and Gram-negative (E. coli) bacterial strains in the presence of various concentrations of the ligands and the complexes ranging from 24 to 100 μg mL⁻¹. No significant antimicrobial activity was found for free mbtt and PPh₃ against any of the strains tested [16]. The half-minimal inhibitory concentration (IC₅₀) values obtained for 1–3 are presented in table 5.

An essentially equal antibacterial activity was found for the three copper(I) halide complexes (1–3), with marginal differentiations of the effectiveness following the order [CuCl(mbtt)]₂⁺ > [CuBr(mbtt)]₂⁺ > [CuI(mbtt)]₂⁺, with half-minimum inhibitory concentration (IC₅₀) values ranging from 36 to 40 μg mL⁻¹ (28.98–36.86 μM) against S. aureus, from 25 to 44 μg·mL⁻¹ (20.13–37.48 μM) against B. subtilis, and from 38 to 46 μg·mL⁻¹ (30.58–39.18 μM) against B. cereus, while the IC₅₀ values for E. coli reached 100 μg·mL⁻¹ (80.50–92.15 μM) or higher. For the time being, a qualitative assessment of these results reveals that 1–3 are less effective bacteriostatics than their recently reported mononuclear counterparts [16], a result that cannot be easily explained in terms of the current knowledge.

### 3.5. Interaction with CT DNA

According to their structure and the nature of their ligands, metal complexes may bind covalently to double-stranded DNA, interact non-covalently with DNA, or induce cleavage of the DNA-helix. In the

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**Figure 3.** Crystal structure of 3. Displacement ellipsoids are shown at the 50% probability level.

| Compound                     | S. aureus (μg mL⁻¹) | B. subtilis (μM) | B. cereus (μM) |
|------------------------------|---------------------|------------------|----------------|
| [CuCl(PPh₃)(mbtt)]₁         | 40 (36.86)          | 35 (32.25)       | 40 (36.86)     |
| [CuBr(PPh₃)(mbtt)]₂         | 40 (34.07)          | 44 (37.48)       | 46 (39.18)     |
| [CuI(PPh₃)(mbtt)]₃          | 36 (28.98)          | 25 (20.13)       | 38 (30.59)     |
case of covalent binding to DNA bases, one or more labile ligands of the complex may be replaced by a nitrogen DNA-base. Complexes that keep their integrity in solution may either induce cleavage of the DNA-helix or interact non-covalently with DNA. Non-covalent interactions of the complexes with DNA include intercalation between the DNA-base pairs (as a result of \( \pi \rightarrow \pi \) stacking interactions of the complex planar aromatic rings and DNA-bases), electrostatic interactions (attributed to Coulomb forces between DNA-phosphate groups and the complex), and groove-binding (external binding assigned to hydrophobic or hydrogen bonding or van der Waals forces) [39].

The interactions of mbtt and 1–3 with CT DNA were investigated directly by UV spectroscopy and viscosity measurements and indirectly via the evaluation of the EB-displacing ability of the compounds as examined by fluorescence emission spectroscopy.

3.5.1. DNA binding with UV spectroscopy
UV spectroscopy is the usual technique employed to study the interaction of compounds with DNA to obtain preliminary information concerning the DNA interaction mode and to calculate the DNA-binding constant (\( K_b \)) of the compounds. More specifically, the UV spectra of a CT DNA solution (1.5–1.75 \( \times 10^{-4} \) M) were recorded in the presence of the compounds (i.e. mbtt and 1–3) at increasing amounts (for different \( r \) values) and vice versa, i.e. the UV spectra of the compounds (1–4 \( \times 10^{-5} \) M) were recorded in the presence of increasing amounts of CT DNA. The existence of an interaction with DNA may induce changes to the CT DNA band located at 258–260 nm in the former study or to the intraligand transition bands in the latter study during the titrations providing, thus, information of the interaction.

The CT DNA band located at \( \lambda_{\text{max}} = 258 \) nm in the UV spectra of a CT DNA solution exhibited in the presence of the compounds a slight hypochromism as representatively shown in the presence of mbtt in figure 4(A). Similar changes were observed in UV spectra of CT DNA in the presence of the other compounds and may reveal the interaction of the compounds with CT DNA through formation of a new compound–DNA conjugate [39].

In the UV spectra of the compounds, an intraligand band is located at 325 nm. In the UV spectra of the compounds, a hypochromism in the range 2.5–9% (table 6) is observed in the presence of CT DNA (representatively shown for 2 in figure 4(B)) while the position of the band does not present any appreciable change. The presence of hypochromism in the UV spectra of the compounds upon addition of CT-DNA could be considered as a hint of the existence of intercalation between the compounds and CT DNA, but the extent of hypochromism observed is not so pronounced in order to come to a safe conclusion concerning the possible complex–DNA interaction mode only from the existing UV spectroscopic data [40]; within this context, DNA viscosity measurements were employed in an attempt to better clarify the interaction mode.

The DNA-binding constants (\( K_b \)) of the compounds (i.e. mbtt and 1–3) (table 6) were determined by the Wolfe–Shimer equation (equation 1) [41] using plots [DNA]/(\( \varepsilon_{\text{a}} - \varepsilon_{\text{b}} \)) versus [DNA] (figure S1). The \( K_b \) constants of the complexes are relatively high and higher than that of free mbtt, especially for 2 and 3 which bear the highest \( K_b \) constants (\( K_{b(2)} = 1.30(\pm 0.25) \times 10^6 \) M\(^{-1} \) and \( K_{b(3)} = 1.06(\pm 0.22) \times 10^6 \) M\(^{-1} \)) among the present compounds. Additionally, the \( K_b \) constants of the compounds are similar (in the case of mbtt and 1) or higher (for 2 and 3) than that of the classical intercalator EB (\( =1.23(\pm 0.07) \times 10^5 \) M\(^{-1} \)) as calculated in our lab [41].

3.5.2. DNA-viscosity measurements
DNA-viscosity measurements in the presence of mbtt and 1–3 were also employed in order to evaluate the interaction of the compounds with CT DNA. Such measurements may further clarify the DNA-interaction mode of a compound, since the relative DNA-viscosity (\( \eta/\eta_0 \)) is sensitive to relative DNA-length changes (\( L/L_0 \)) [42] according to the equation \( L/L_0 = (\eta/\eta_0)^{1/3} \) [42]. Within this context, the viscosity of a CT DNA solution (0.1 mM) was monitored upon addition of increasing amounts of the compounds (up to the value of \( r = 0.35 \)). A considerable increase in the relative DNA-viscosity was observed in the presence of increasing amounts of the compounds (figure 5), and especially in the presence of 2 and 3.
In general, the intercalation of a compound between the DNA base pairs to DNA may induce an increase in the separation distance of the DNA-base pairs close to the intercalation sites so to host the inserting compound and a subsequent increase of the relative DNA-length resulting in an increase in relative DNA-viscosity; the magnitude of the increase is usually in accord to the strength of the interaction. When a compound binds to DNA-grooves via a partial or non-classic intercalation (i.e. electrostatic interaction or external groove-binding) a bend or kink in the DNA-helix may occur and the relative DNA-length may present a slight shortening; in such a case, the DNA-viscosity may show a slight decrease or may remain unchanged [43].

Therefore, the increase in the relative DNA-viscosity observed in the presence of compounds may be attributed to the existence of intercalation between DNA and the compounds [32, 44–46]. The existing conclusion of intercalation may clarify the UV-spectroscopic findings. In particular, a higher extent of intercalation may be expected for 2 and 3 which present the higher affinity for CT-DNA (higher $K_b$ constants).

**Figure 4.** (A) UV spectra of CT DNA ([DNA] = (1.75×10$^{-4}$ M) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the absence or presence of mbtt at increasing amounts. The arrow shows the changes upon increasing amounts of the compound. (B) UV spectra of DMSO solution of 2 (2 × 10$^{-3}$ M) in the presence of increasing amounts of CT DNA (′ = [DNA]/[compound] = 0–0.8). The arrow shows the changes upon increasing amounts of CT DNA.

**Table 6.** UV spectral features of the interaction of mbtt and 1–3 with CT DNA. UV-band ($\lambda$, nm) (percentage of the observed hyper-/hypo-chromism ($\Delta\lambda_\lambda$/$\lambda_\lambda$ (%)), blue-/red-shift of the $\lambda_{\max}$ (Δ$\lambda$, nm)) and DNA-binding constants ($K_b$, M$^{-1}$).

| Compound                  | Band (nm) (ΔA/$\lambda_\lambda$(%)$^a$, Δ$\lambda$(nm))$^b$ | $K_b$ (M$^{-1}$) |
|---------------------------|-------------------------------------------------------------|-----------------|
| mbtt                      | 324 (−5, 0)                                                | 9.57 (±0.12) × 10$^4$ |
| [CuCl(PPh$_3$)(mbtt)], 1  | 325 (−2.5, 0)                                              | 1.64 (±0.30) × 10$^5$ |
| [CuBr(PPh$_3$)(mbtt)], 2  | 325 (−9, 0)                                                | 1.30 (±0.25) × 10$^6$ |
| [Cul(PPh$_3$)(mbtt)], 3   | 325 (−8, 0)                                                | 1.06 (±0.22) × 10$^6$ |

$^a$ + ” denotes hyperchromism, “−” denotes hypochromism.

$^b$ + ” denotes red-shift, “−” denotes blue-shift.
3.5.3. EB-competitive studies with fluorescence emission spectroscopy

EB is a well-known DNA-intercalator, since it can intercalate between the DNA-base pairs through the planar EB-phenanthridine ring. EB is considered an indicator of DNA-intercalation, since the EB–DNA conjugate exhibits an intense fluorescence emission band at 592–593 nm when excited at 540 nm [32] and the addition of another intercalating compound may induce significant quenching of this EB–DNA emission band [32, 47].

**Figure 5.** Relative viscosity \((\eta/\eta_0)^{1/3}\) of CT DNA (0.1 mM) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the presence of mbtt and 1–3 at increasing amounts \((r = [\text{compound}]/[\text{DNA}])\).

**Figure 6.** (A) Fluorescence emission spectra \((\lambda_{ex} = 540 \text{ nm})\) for EB–DNA ([EB] = 20 µM, [DNA] = 26 µM) in buffer solution in the absence and presence of increasing amounts of 1 (up to the value of \(r = 0.14\)). The arrow shows the changes of intensity upon increasing amounts of 1. (B) Plot of EB-DNA relative fluorescence emission intensity (%\(I/I_0\)) at \(\lambda_{em} = 592 \text{ nm}\) vs. \(r = [\text{compound}]/[\text{DNA}]\) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0) in the presence of mbtt and 1–3 (quenching up to 34.0% of the initial EB–DNA fluorescence for mbtt, 27.7% for 1, 31.0% for 2 and 28.5% for 3).
The EB–DNA conjugate was prepared after 1 h pretreatment of EB ([EB] = 20 μM) and CT DNA ([DNA] = 26 μM) in buffer solution (150 mM NaCl and 15 mM trisodium citrate at pH 7.0). The fluorescence emission spectra (λ_{ex} = 540 nm) of the EB–DNA solution were recorded in the presence of increasing amounts of mbtt and 1–3 (representatively shown for 1 in figure 6(A)), showing that the addition of the compounds in the EB–DNA solution resulted in moderate-to-significant quenching of the emission band of the DNA–EB system at 592 nm (figure 6(B)). The final quenching (ΔI/I_0) is in the range 66–72.3% of the initial EB–DNA fluorescence (table 7) and may reveal the ability of the compounds to displace EB, in competition for DNA-intercalating sites. Therefore, the existence of intercalation of the compounds to CT DNA may be indirectly concluded [44–46, 48].

The observed quenching of the EB–DNA fluorescence is in good agreement (R = 0.99) with the linear Stern–Volmer equation (equation 2) [26] as indicated in the corresponding Stern–Volmer plots (figure S2). The KSV constants of the compounds (table 7) are moderate-to-high verifying their binding affinity for DNA. Complexes 1–3 present higher KSV constants than free mbtt, and 3 has the highest KSV constant (=1.12(±0.04) × 10^6 M\(^{-1}\)) among the compounds. Since the fluorescence lifetime of EB-DNA system (τ_0) equals to 23 ns [33], the quenching constants of the compounds (k_q) were calculated with equation 3 (table 7). The k_q constants are significantly higher than 10^{10} M\(^{-1}\)s\(^{-1}\) suggesting, thus, that the quenching of the EB-DNA fluorescence takes place via a static mechanism [48, 49].

4. Conclusion

The coordination behavior of N-methylbenzo-thiazole-2-thione toward [CuX(PPh_3)]_4 has been explored. In the doubly bridged dicopper(I) species obtained, N-methylbenzothiazole-2-thione coordinates exclusively via the “soft” exocyclic sulfur either in a μ_2-S bridging mode (in the case of X = I), or as terminal ligand, with the halide (Cl or Br) participating in bridge formation. Moderate antibacterial activity has been found for 1–3, which proved to be less effective bacteriostatics than the corresponding mononuclear ones. Intercalation is the most possible interaction mode between the dinuclear complexes 1–3 and CT-DNA as indicated especially by DNA viscosity experiments and EB-displacement studies.

Supplementary material

CCDC 1472508 – 1472510 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44–1223/336–033; E-mail: deposit@ccdc.cam.ac.uk].

Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 7. Data of the EB-competitive studies of mbtt and 1–3. Percentage of EB-DNA fluorescence quenching (ΔI/I_0, %), Stern-Volmer constants (K_{SV}, M\(^{-1}\)) and quenching constants (k_q, M\(^{-1}\)s\(^{-1}\)).

| Compound                     | ΔI/I_0 (%) | K_{SV} (M\(^{-1}\)) | k_q (M\(^{-1}\)s\(^{-1}\)) |
|------------------------------|------------|---------------------|---------------------------|
| mbtt                         | 66.0       | 9.63(±0.28) × 10^4  | 4.19(±0.12) × 10^{12}     |
| [CuCl(PPh_3)(mbtt)], 1        | 72.3       | 9.73(±0.18) × 10^5  | 4.23(±0.08) × 10^{13}     |
| [CuBr(PPh_3)(mbtt)], 2        | 69.0       | 8.76(±0.20) × 10^5  | 3.81(±0.09) × 10^{13}     |
| [Cui(PPh_3)(mbtt)], 3         | 71.5       | 1.12(±0.04) × 10^6  | 4.87(±0.15) × 10^{13}     |
