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The thiol-based reduction of Bi(V) and Sb(V) anti-leishmanial complexes

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ABSTRACT

Low molecular weight thiols including trypanothione and glutathione play an important function in the cellular growth, maintenance and reduction of oxidative stress in *Leishmania* species. In particular, parasite specific trypanothione has been established as a prime target for new anti-leishmania drugs. Previous studies into the interaction of the front-line Sb(V) based anti-leishmanial drug meglumine antimoniate with glutathione, have demonstrated that a reduction pathway may be responsible for its effective and selective nature. The new suite of organometallic complexes, of general formula $[MAr_3(O_2CR)_2]$ (M = Sb or Bi) have been shown to have potential as new selective drug candidates. However, their behaviour towards the critical thiols glutathione and trypanothione is still largely unknown. Using NMR spectroscopy and mass spectrometry we have examined the interaction of the analogous Sb(V) and Bi(V) organometallic complexes, $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **S1** and $[BiPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **B1**, with the trifluoroacetate (TFA) salt of trypanothione and L-glutathione. In the presence of trypanothione or glutathione at the clinically relevant pH of 4–5 for *Leishmania* amastigotes, both complexes undergo facile and rapid reduction, with no discernible difference. However, at a higher pH (6–7), the complexes behave quite differently towards glutathione. The Bi(V) complex is again reduced rapidly but the Sb (V) complex undergoes slow reduction over 8 h ($t_{1/2}$ = 54 min.) These results give the first insights into why the highly oxidising Bi(V) complexes display low selectivity in their cytotoxicity towards leishmanial and mammalian cells, while the Sb(V) complexes show good selectivity.

1. Introduction

Leishmaniasis is a devastating tropical disease caused by parasites of the *Leishmania* species [1]. Millions of people are currently at risk of developing the disease, with upwards of 40,000 fatalities due to a lack of adequate treatment options [2–4]. Front-line treatments have relied almost exclusively on the use of the pentavalent antimonials sodium stibogluconate and meglumine antimoniate since the beginning of the second World War [5]. Despite this, little is known and understood about their mechanism of action and selective toxicity. Our current understanding is focused on a reductive pathway as the primary mechanism of action, with Sb(V) acting as a pro-drug where it is biochemically reduced *in vivo* to the more bioactive Sb(III) form [6]. This hypothesis is further supported by the apparent lack of toxicity of Sb(V), and high toxicity of Sb(III) complexes to both stages of the parasite of different *Leishmania* species [7–9]. Conversely, it has been observed that Pentostam™ (sodium stibogluconate) has a parasiticidal effect on axenic amastigotes [10].

The redox chemistry associated with current Sb(V) treatments occurs preferentially in the low pH environment of the amastigote (pH 4–5), and is believed to be facilitated by low molecular weight thiols such as glutathione and trypanothione, the latter being parasite specific (Fig. 1) [11,12]. Unlike most organisms, *Leishmania* species can conjugate the tripeptide glutathione, and the polyamine spermidine to form trypanothione. This compound, which is the most abundant low molecular weight thiol inside the *Leishmania* species, is essential for the growth and survival of the parasite, providing a unique intracellular reducing environment. As with glutathione in mammalian cells, trypanothione can act as a redox scavenger and anti-oxidant, and hence is a crucial part of the defensive mechanisms employed by *Leishmania* parasites in preventing oxidative stress. The fact that trypanothione, and its related enzymes, are not present in mammalian cells makes them attractive

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targets for current and future anti-leishmanial drugs. Indeed, Sun and co-workers have demonstrated the rapid reduction of Sb(V) (as sodium stibogluconate) to Sb(III) under both mildly acidic and neutral conditions by trypanothione [12,13]. In contrast, the reduction of this compound is negligible in the presence of the mammalian analogue glutathione, under the same conditions. In a separate study, the same group has shown that Sb(III) (as antimony tartrate) can form a novel complex with trypanothione, through binding of the two sulfur atoms on the cysteine residues, and the oxygen of a water molecule to the metal centre [14]. In addition to their high affinity for trypanothione, the enzyme responsible for keeping it in its reduced form, trypanothione reductase, is also a major target site for current and future anti-leishmanial drugs, including Sb(III) complexes. [15] [16,17].The solid state crystal structure of Sb(III) with reduced trypanothione reductase has been obtained, revealing the ability of the metal to directly coordinate to the catalytic centre of the enzyme, and in particular with the Cys52, Cys57, Thr335 and His461' residues [17].

Various mechanistic studies for pentavalent antimonials have also been conducted using glutathione. Frezard et al. studied the interaction of Glucantime™ (meglumine antimoniate) with glutathione at different temperatures and pH values, mimicking both mammalian physiological conditions and that of the parasite [11], concluding that GSH promotes the reduction of Sb(V)/Sb(III) at an acidic pH, with elevated temperatures increasing the rate [11]. This study also concluded that the reduction should not occur in the neutral pH of the host cell cytosol despite a high concentration of GSH, but could be facilitated in more acidic macrophage organelles [11,18]. Sun et al. determined Sb(III) to have a high binding affinity to glutathione, indicating that thiols were the preferential biological target for Sb(III) [19].

Further studies by Frezard et al. focused on other biologically relevant thiols such as cysteinyl-glycine, cysteine, as well as trypanothione. Reduction was monitored using both complexed Sb(V), as meglumine antimoniate, and 'free' antimoniate as $K[Sb(OH)_6]$) [20]. Similar to their previous study, they noted that the rate of Sb(V/III) reduction increased at lower pH and higher temperatures, and concluded that complexed *vs* un-complexed antimoniate underwent reduction at different rates [20]. However, studies by Yan et al. determined that the reduction of meglumine antimoniate by glutathione and other mammalian moieties were too slow to be biologically significant and that the parasite specific trypanothione would constitute the greatest reductive activity [12].

Recent work into the design of new antimony and bismuth based anti-leishmanial drugs has focused on organometallic carboxylates; as a way of decreasing toxicity while increasing compound lipophilicity to allow for possible oral delivery and uptake through the stomach, something not possible with the current hydrophilic Sb(V) drugs [21–30]. Despite promising *in vitro* efficacy and cell selectivity, the stability of these $[MAr_3(O_2CR)_2]$ (M = Sb or Bi) complexes has so far only been studied in NMR solvents and in cell media. There have been no investigations into the chemical behaviour of these organometallic complexes in the presence of either glutathione or trypanothione. Herein, we present a NMR spectroscopic study on the interaction of two previously synthesised *tris*-phenyl M(V) acetato complexes (M = Sb or Bi); $[SbPh_3(O_2CCH_2R)_2]$ **S1** and $[BiPh_3(O_2CCH_2R)_2]$ **B1** (R = *p*-tolyl), with trypanothione and L-glutathione, in an attempt to shed light on their stability, redox activity, and reactivity [24].

2. Experimental

2.1. General

L-Glutathione was purchased from Oakwood chemicals without the need for further purification. *p*-Tolyl acetic acid, 70% tert-butyl hydroperoxide (Luperox ™) and triphenyl antimony were purchased from Sigma Aldrich. All remaining solvents and reagents were purchased from either Sigma Aldrich or Merck. ^{1}H NMR spectra were recorded on a Bruker Avance DRX600 spectrophotometer (600 MHz), chemical shifts were references to the appropriate deuterated solvent, D_2O or d_6-DMSO. Melting point analysis was conducted in open end capillary tubes, on a digital Stuart Scientific melting point apparatus SMP10. High-resolution ESI mass spectroscopy (high-res ESI MS) was run on a Micromass Platform QMS spectrometer, with an electrospray source and a cone voltage of 35 eV.

2.2. Synthesis of trifluoroacetate (TFA) salt of trypanothione

The synthesis of the TFA-salt of trypanothione (TSH) was achieved using methods as first detailed by Antoniou et al. [31]. However, in contrast to this publication, that used the highly toxic diazomethane as a precursor, the key diester intermediate **1** was obtained in three-steps using much more benign reagents (Fig. 2). The full characterization of the intermediates obtained in the synthesis of diester **1** are available in the supporting information (SI Figs. S1 –S20). Briefly, to the commercially available L-glutathione (2.0 g, 6.5 mmol) in DCM (15 mL) was added trityl chloride (2.0 g, 7.0 mmol) portion-wise. The resulting solution was stirred overnight, before being concentrated under *vacuo* to yield a white solid that was taken forward to the next step of the synthesis without the need for further purification. Methanol (60 mL) was then used to dissolve this compound (S-trityl protected glutathione **2** (4.0 g, 7.3 mmol)), before a catalytic amount of *p*-TSA (277 mg, 1.5 mmol) was added. The reaction was stirred at reflux overnight before the resulting solution was concentrated under *vacuo*. The crude white solid that ensued was taken forward to the next step of the synthesis without the need for further purification. In the final step to obtain diester intermediate **1**, S-trityl protected glutathione ester **3** (1.6 g, 2.8 mmol) was first dissolved in DCM (40 mL), before being cooled to 0 °C. Once cooled, a solution of trityl chloride (850 mg, 3.0 mmol) and triethylamine (0.77 mL, 5.5 mmol) in DCM was added dropwise. A clear

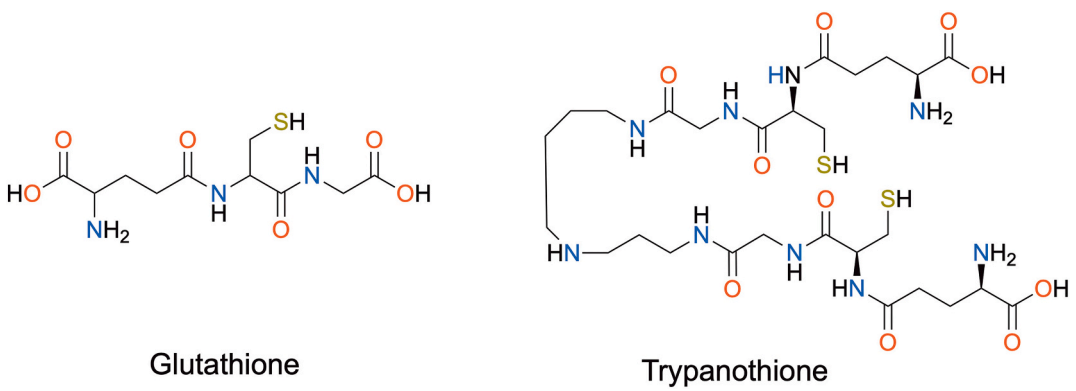

Fig. 1. Chemical structures of glutathione and trypanothione.

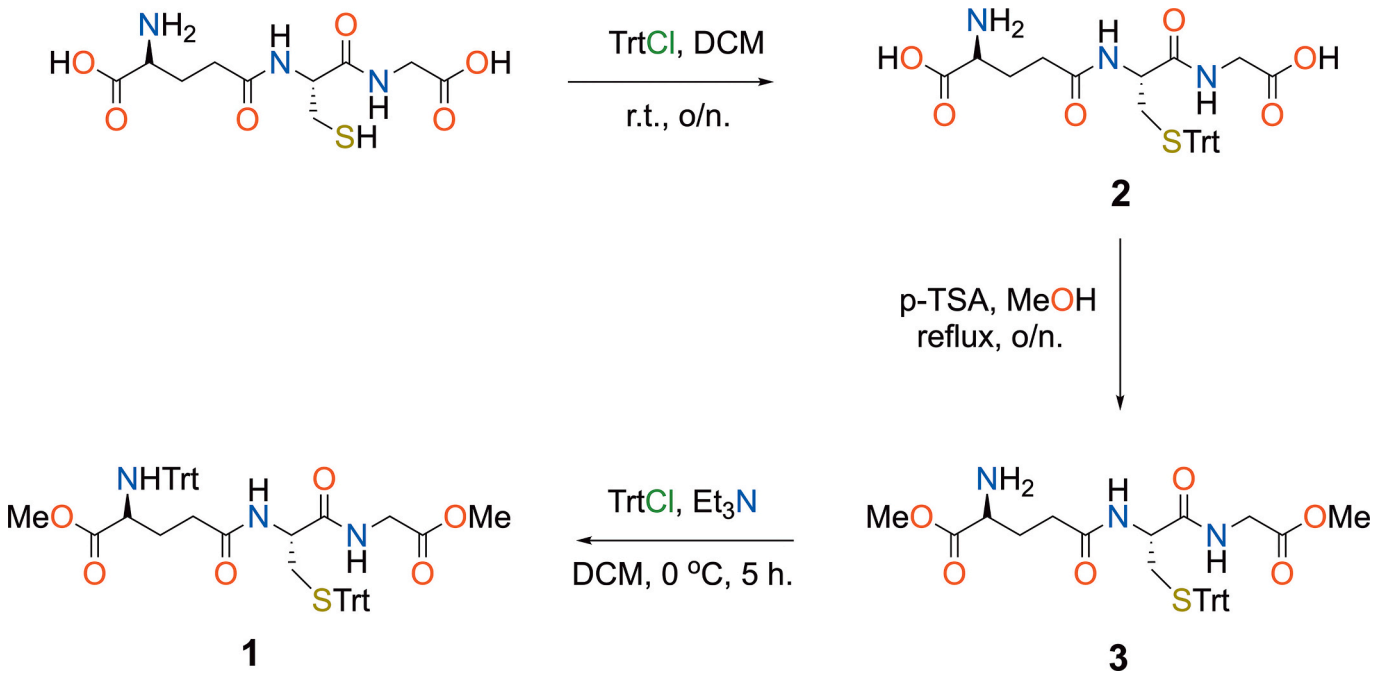

Fig. 2. Alternative synthesis to diester **1**; a key intermediate required in the synthesis of trypanothione.

glassy solid was obtained following the reaction time, and work-up procedure as detailed by Antoniou et al. [31].The synthesis of N-boc protected spermidine [32] and its subsequent involvement in the synthesis of trypanothione followed methods previously detailed in the literature. All structural characterization of the intermediates isolated during this synthesis matched those previously reported in the literature [31,32].

The formation of TFA-trypanothione was confirmed by ^{1}H, ^{13}C and ^{19}F NMR spectroscopy, all of which matched the spectra of those previously reported in the literature (see SI, Figs. S1–S4 for details). The formation of TFA-trypanothione was also confirmed by high-res ESI MS, whilst the purity of the compound, was analysed by RP-HPLC on a C-8 column using a linear gradient from 5% to 40% B over 20 min, where A = 0.1% TFA in water and B = 0.1% TFA in MeCN, at a flow rate 0.9 mL/min and monitored at 214 nm: t_R = 8.31 min.

2.3. Synthesis of $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$, **S1** and $[BiPh_3(O_2CCH_2(C_6H_4CH_3))_2]$, **B1**

Both complexes were prepared as previously described [24]. Briefly: 0.5 mmol of $BiPh_3$ or $SbPh_3$ was dissolved in toluene, and to this solution two equivalents of 70% tBuOOH was added. 1.0 mmol of *p*-tolylacetic acid was then added and the reaction stirred for approximately an hour. The solution was filtered and left to stand yielding both complexes as crystalline solids. The complexes were identified by ^{1}H NMR and melting point analysis [24].

2.4. ^{1}H NMR spectroscopic study

Both complexes were made into 10 mM stocks in 1 mL of d_6-DMSO. 100 μL of this solution was placed into an NMR tube and the contents frozen in liquid nitrogen at −80 °C. To this frozen tube, 3.5 equivalents of trypanothione, or 7 equivalents of glutathione in 400 μL of 1:1 D_2O/DMSO mix was added and the contents frozen once again. For the low pH studies, spectra were recorded one after another for upwards of 1 h, and every 2 h thereafter. For the higher pH glutathione study, spectra were recorded every half hour for Sb and one after the other for upwards of an hour for Bi. The half-life of the Sb complex was then calculated by proton integration ratio of the original complex to the formation of $SbPh_3$. The decomposition graph was obtained using Microsoft excel and the corresponding half-life derived from the logarithmic equation produced.

2.5. Mass spectrometry

For the studies conducted at low pH, aliquots were taken directly from the NMR tubes, diluted and then analysed by high-res ESI mass spectroscopy. For the glutathione study conducted at a higher pH (6–7), both complexes were reacted with an excess of glutathione in a 1:1 mix of degassed H_2O:EtOH. The mixture was heated overnight before filtration. Both the solid and filtrate were analysed in degassed H_2O on a Micromass Platform QMS spectrometer.

3. Results and discussion

Complexes **S1** and **B1**, $[MPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ (**S1**, M = Sb; **B1**, M = Bi) (Fig. 3), were synthesised and purified as previously described

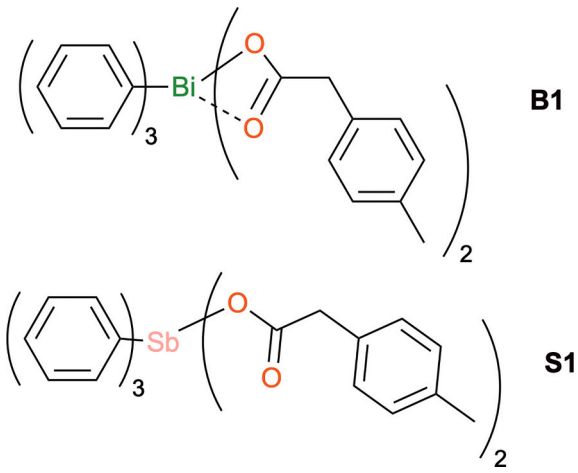

Fig. 3. Chemical structures of complexes $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$, **S1** and $[BiPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **B1** analysed in this study.

[23]. The composition of the crystalline products was confirmed using ^{1}H NMR spectroscopy and melting point analysis, showing them to be the desired complexes.

3.1. Reduction of complexes B1 and S1 with TFA-trypanothione (pH 4–5)

Our original studies on these two complexes showed **B1** to have little selectivity for the *Leishmania* parasite cells over mammalian cells, and that **S1** was much more selective [24]. As such, we anticipated that the Bi complex, **B1**, would display higher reactivity towards both trypanothione and glutathione, originating from the significant difference in reduction potential between Bi(V/III) and Sb(V/III) of 2.00 and 0.605 V respectively [33].

Though the promastigote form of the parasite is the initial source of infection, this form is unable to proliferate in mammalian hosts and only spends a small amount of time in the bloodstream before being taken up by macrophages pending an immune response. The clinically relevant form of the parasite is the amastigote, and it is this which is the target of interest for novel anti-leishmania drugs, as it hides and proliferates within the immune cells of the mammalian host [17,33]. *In vivo*, the amastigote residues in an acidic environment of *ca.* pH 4–5.5 [18,34]. To mimic this in our study, we prepared the TFA salt of trypanothione, which gives a final pH of between 4 and 5, and expected this compound to have the same chemical effect on the organometallic complexes as trypanothione itself.

In our investigation, both complexes were first dissolved in d_6-DMSO at a stock solution concentration of 10 mM. Sonication was used to assist the dissolution of the bismuth complex $[BiPh_3(O_2CCH_2(C_6H_4CH_3))_2]$, **B1**. A known concentration of **B1** and **S1** were then placed into an NMR tube and the contents frozen. To this, a solution of the TFA-trypanothione salt (3.5 equivalents), at pH 4–5 in D_2O/d_6-DMSO (1:1), was added to the frozen tube and the contents immediately frozen once again to inhibit any reaction from occurring. On melting and mixing of the components, the kinetics of Sb(V) and Bi(V) reduction by the TFA-trypanothione were studied by ^{1}H NMR spectroscopy.

The ^{1}H NMR spectrum acquired immediately after mixing revealed that both **S1** and **B1** are rapidly reduced by trypanothione at this particular pH. Both spectra demonstrate that the Sb(V) and Bi(V) complexes are immediately reduced, with the presence of $SbPh_3$ (7.29–7.34 ppm) and $BiPh_3$ (7.66, 7.35 and 7.27 ppm), and the parent *p*-tolylacetic acid (7.07, 3.47 and 2.20 ppm) clearly visible in their respective ^{1}H NMR spectra (Figs. 4 and 5). In addition, the β-protons of the cysteine residue of trypanothione at 2.70 and 2.77 ppm (see SI Figs. S1–S4 for NMR spectrum of trypanothione) are reduced in intensity, with a new resonance detected at approximately 3.10 ppm. The resonance relating to the α-proton of the cysteine residue is also clearly reduced in intensity, with a new resonance seen at approximately 4.30 ppm. Both these phenomena are indicative of the formation of the oxidised form of trypanothione, as well as the possible formation of a metal (Sb(III) or Bi(III)) trypanothione species, as was previously demonstrated by Sun et al. [12–14]

In order to confirm the presence of MPh_3, *p*-tolylacetic acid, oxidised trypanothione, and any related metal trypanothione complex, aliquots were taken from respective NMR tubes and analysed by high-res ESI-MS. In both cases, the presence of oxidised trypanothione was clearly identified (722.30619 – $[M + H]^+$) (see SI Fig. S17), however no other species that were present in the ^{1}H NMR spectra, were detected using this method.

3.2. Reduction of complexes B1 and S1 with glutathione (pH 4–5)

The determination that **S1** and **B1** are rapidly reduced (time '0') in the presence of trypanothione at pH 4–5, provides support for the mechanism by which both Sb(V) and Bi(V) exert a toxic effect on the amastigotes. The remaining question is that of selectivity (selectivity index for **S1** is >9, no toxicity towards mammalian cells was observed at the highest concentration of 100 μM, and for **B1** is 6.3, high degree of both parasite and mammalian toxicity) and toxicity towards the mammalian host cells. To answer this, we turned to study the impact of glutathione as the dominant reducing thiol peptide in mammalian cells, but of lower concentration in leishmanial cells. As was the case with

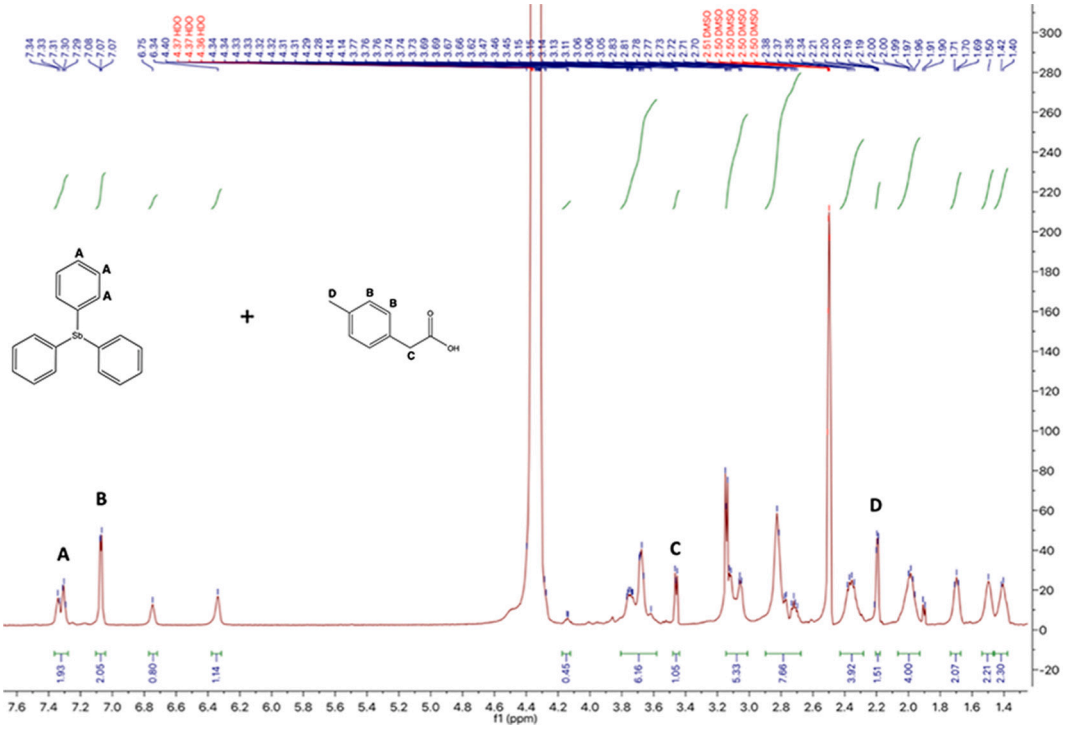

Fig. 4. ^{1}H NMR spectra of complex $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **S1** with an excess of TFA-trypanothione in d_6-DMSO/D_2O 1:1 mix at '0 min'. The formation of $SbPh_3$ and *p*-tolylacetic acid have been highlighted.

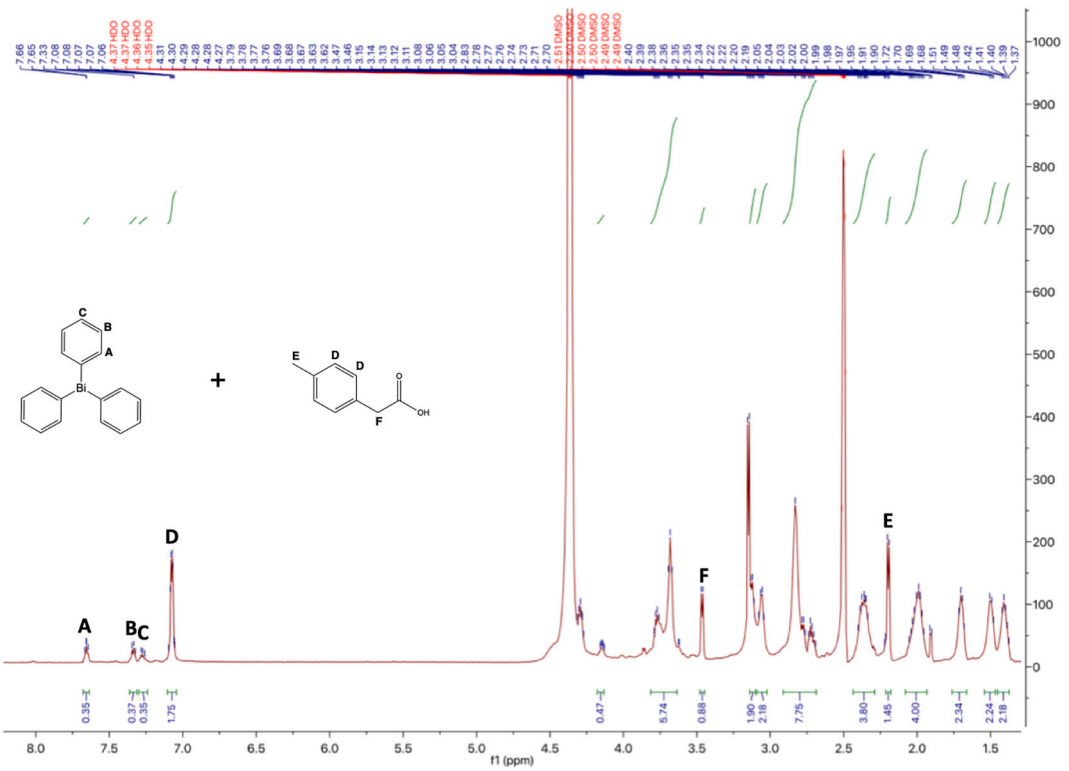

Fig. 5. ^{1}H NMR spectra of complex [$BiPh_3(O_2CCH_2(C_6H_4CH_3))_2$] **B1** with an excess of TFA-trypanothione in d_6-DMSO/D_2O 1:1 mix at '0 min'. The formation of $BiPh_3$ and *p*-tolylacetic acid have been highlighted.

trypanothione, under the same conditions the ^{1}H NMR spectra both indicate that **S1** and **B1** are again rapidly reduced, with MPh_3 and *p*-tolylacetic acid observed in the respective NMR spectra at time point '0' (see SI Figs. S7–S10).

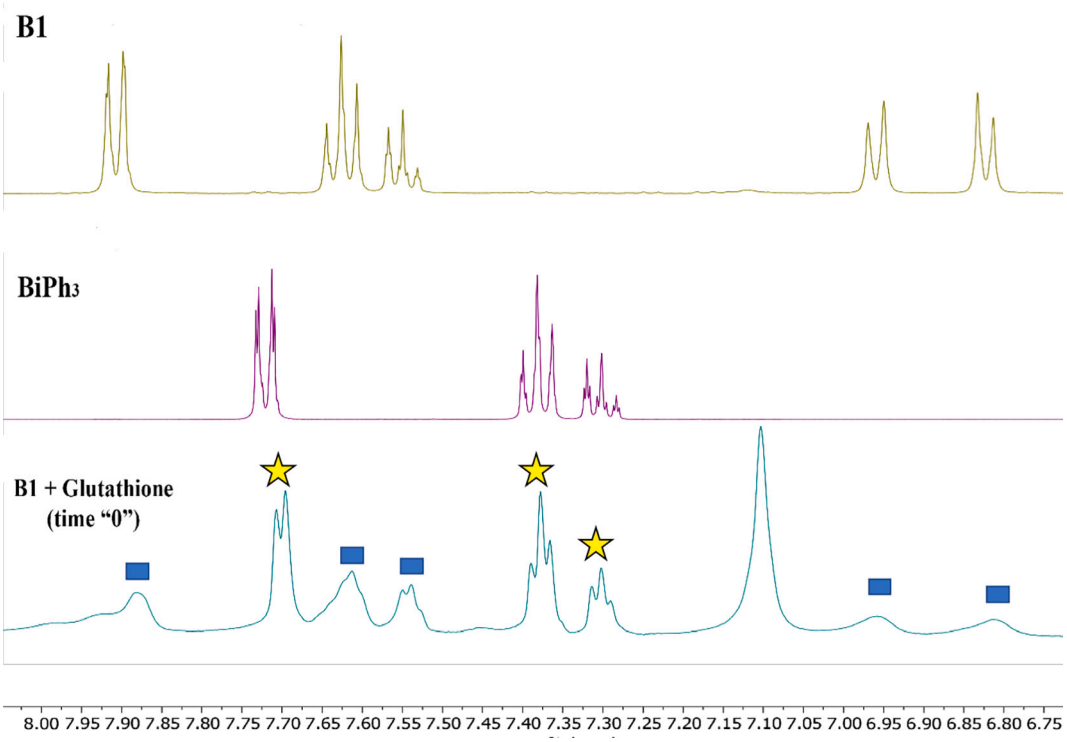

Fig. 6. Overlay of ^{1}H NMR spectra of [$BiPh_3(O_2CCH_2(C_6H_4CH_3))_2$] **B1**, $BiPh_3$ and **B1** + GSH (t = "0"), highlighting the presence of both **B1** and $BiPh_3$ in the reaction mixture at time point '0'. At t = "0", peaks corresponding to $BiPh_3$ are donated by blue squares whilst those corresponding to **B1** are labelled with yellow stars. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Stability of complexes B1 and S1 in acidic pH

To determine the impact solely of solution pH on the stability and reaction kinetics, a D_2O/DMSO solution of TFA at pH 4–5 was used to dissolve complexes **S1** and **B1**, and ^{1}H NMR spectra recorded at set intervals (0, 0.5, 1, 2, 4, 8, 16 and 24 h) for up to 24 h. The spectra showed no changes in splitting or chemical shifts over the 24 h period, indicating both complexes to be stable in the acidic solution (see SI Figs. S11-S14).

3.4. Reduction of complexes B1 and S1 with glutathione (pH 6–7)

Studies have shown that the reduction of Sb(V) complexes is highly pH dependent. The pH of normal mammalian cells is close to 7.0 with glutathione present in concentrations of 5–10 mM and predominantly in the reduced (GSH) form. Having demonstrated that glutathione at a pH of 4–5 can rapidly reduce both **S1** and **B1**, the next part of the study was to establish whether the same was true at neutral pH consistent with healthy mammalian cells.

After following the same reaction procedure, the NMR spectra obtained immediately after the addition of glutathione to the complexes (time '0'), indicated that the bismuth complex **B1** reacts at a much faster rate than the antimony complex **S1**. Complex **B1** was completely reduced in less than 5 min, with the reaction occurring immediately on mixing (Figs. 6 and S15).

For **S1** the reduction reaction was significantly slower. The decomposition rate was calculated by measuring the changing ratio of a signal belonging to **S1**, at 7.92 ppm, against an increasing signal from newly appearing $SbPh_3$, at 7.33 ppm. From this a half-life of 54 min. was determined for the reduction of **S1** by glutathione at pH 6–7 (Fig. 7). Of interest though is the fact that the reduction of **S1** is faster than that observed by Frezard et al. for meglumine antimoniate [11].

As the concentration of **S1** decreases, signals for $SbPh_3$ and *p*-tolylacetic acid appear (Fig. 8). At the conclusion of the reaction a new set of signals are also present that correspond to the formation of oxidised glutathione (GSSG), which was also observed in the spectrum for the reduction of **B1** (see SI Fig. S5).

As an excess of GSH (six equivalents) was used in each reaction the determination of stoichiometry is difficult. GSH has been observed to react with DMSO to form GSSG over time [30] making it difficult to accurately determine the ratio produced in the NMR. Previous studies by Frezard et al. deduced that the Sb(V) meglumine antimonate reacted with five equivalents of glutathione to give the $Sb(GS)_3$ complex and the disulfide GSSG. However, formation of $Sb(GS)_3$ was not proven.

To determine whether $Sb(GS)_3$ is formed on reduction of the organometallic complex **S1**, a 1:3 mixture of **S1** and glutathione was reacted in degassed 1:1 EtOH/H_2O. This suspension was heated to 60 °C and allowed to stir for 24 h. On filtration, the captured solid and the solid material obtained from the filtrate on removal of the ethanol and water were analysed following dissolution in degassed D_2O (see SI Fig. S6). Comparison of the NMR spectra with that obtained for $Sb(GS)_3$ by Sun et al. showed no formation of the $Sb(GS)_3$ in the reduction of **S1** [19]. Mass spectrometry on this sample and others prepared using differing stoichiometries also showed no evidence of the formation of $Sb(GS)_3$. For compound **S1**, ESI-MS was however able to identify the free *p*-tolylacetic acid, and residual complex **S1** (see SI Fig. S16). In contrast, the reaction of the bismuth complex **B1** with glutathione did result in the formation of $Bi(GS)_3$ with the $Bi(GS)_2^+$ fragment observed at *m/z* $[821.1]^+$ (Fig. 9). This may indicate that there are significant differences in their mammalian biochemistry [23,24].

The glutathione redox cycle is an efficient system for the detoxification of oxidising species within mammalian cells [35,36]. GSH is oxidised to GSSG by xenobiotic oxidising species, rendering them harmless. GSSG is then reduced back to its anti-oxidant GSH form by glutathione reductases, allowing GSH to further interact with potentially damaging species [37,38]. As **B1** was found to form the $Bi(GS)_3$ complex, it is possible this may contribute to the increased cytotoxicity that was previously observed [23,24]. Bioavailable GSH would bind strongly to the bismuth centre, disallowing interactions with oxidising species and halting the redox cycle [32]. In contrast to this, previous studies have indicated that the organometallic Sb(V) analogues exhibit little to no activity towards the mammalian controls [23,24]. Though **S1** induces oxidation of GSH to GSSG (subsequently reducing to $SbPh_3$ and the free *p*-tolylacetic acid), there is no evidence of binding to GSH upon its reduction. Therefore, the GSSG produced could undergo enzymatic reduction back to GSH without inhibition of its biological processes.

This decomposition differs from the predicted interaction of

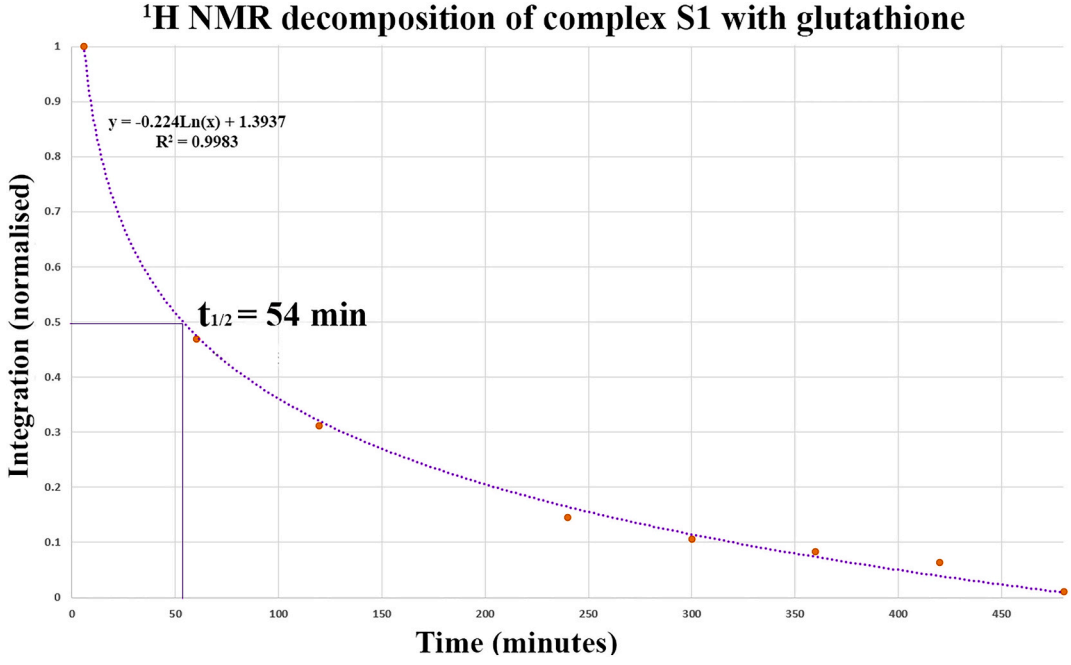

Fig. 7. ^{1}H NMR decomposition graph of $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **S1**, with an excess of GSH in d_6-DMSO:D_2O. Readings were taken hourly on a 600 MHz Bruker NMR spectrometer at 25 °C. A decreasing signal of the original complex was compared to an increasing signal of $SbPh_3$ and a proton ratio obtained.

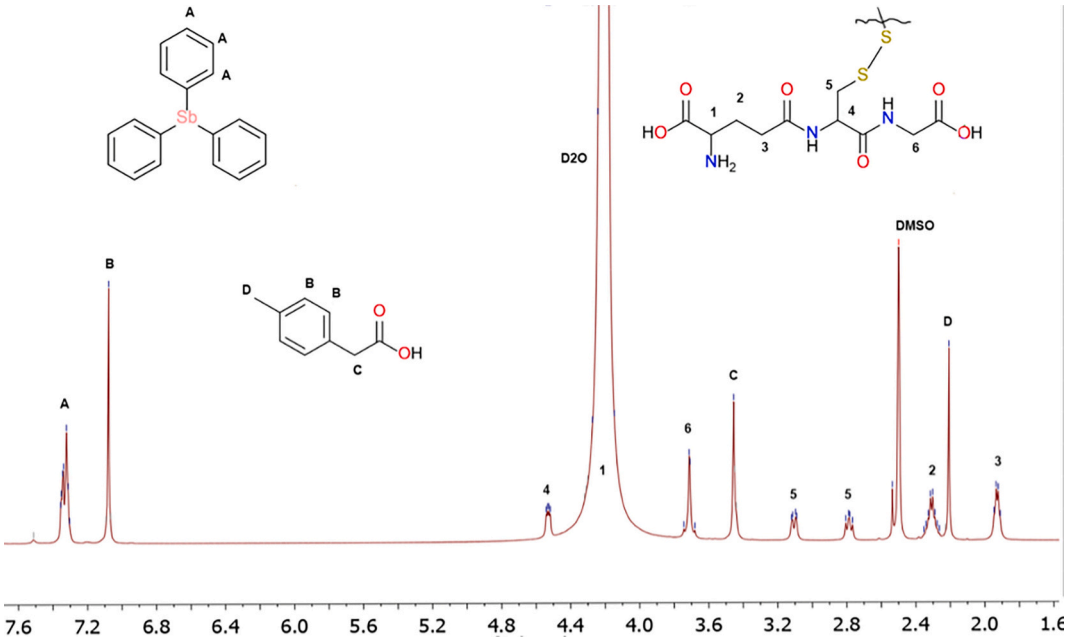

Fig. 8. ^{1}H NMR spectra of complex $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ **S1** with an excess GSH in d_6-DMSO/D_2O 1:1 after 24 h. The formation of $SbPh_3$, *p*-tolylacetic acid and GSSG have been highlighted.

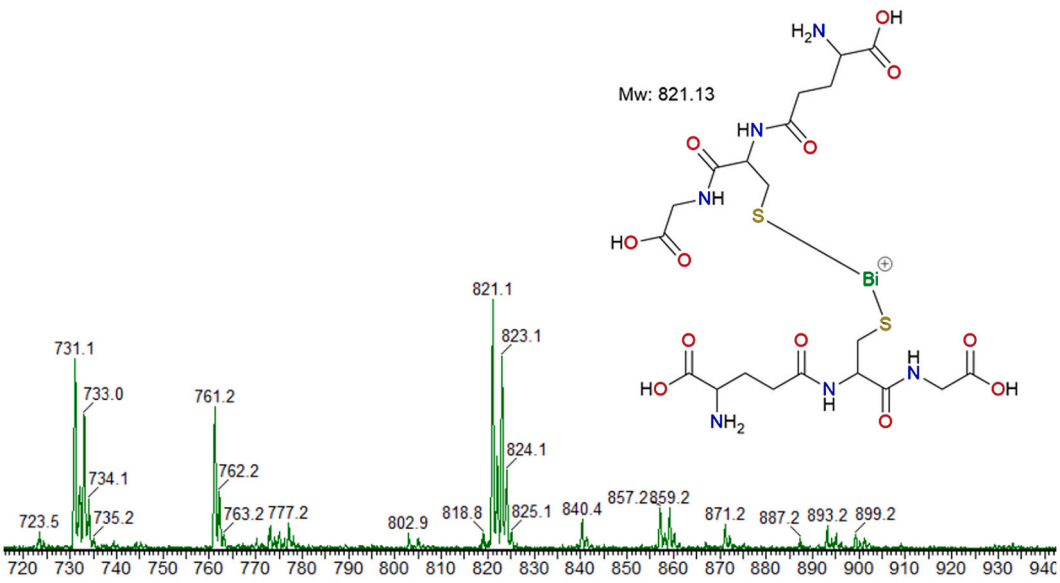

Fig. 9. Positive mode ESI-MS of the reaction of **B1** with glutathione, highlighting the formation of $Bi(Gs)_3$ through the identification of the fragment $[Bi(Gs)_2]^+$.

complexed Sb(V) by Frezard et al. Studies into the interaction of meglumine antimoniate and un-complexed antimonate concluded that the un-complexed antimoniate reduced at a rate that was 8-fold quicker than the complexed meglumine antimoniate [20]. Frezard et al. theorised that due to this, complexed antimony slows down the rate of reduction by blocking interaction with thiol containing compounds. Therefore, this slower reaction rate was attributed to the dissociation of the antimony being the rate limiting step [20].

Nevertheless, the distinctly different chemical and physical properties of meglumine antimoniate as compared to complex **S1** may contribute to the large differences in reaction rate. Complex **S1** is an organometallic compound with three aryl rings and two carboxylate ligands, the solubility of which in aqueous media is limited with a calculated cLogP of 8.14 [39]. Conversely, being highly hydrophilic meglumine antimoniate has a cLogP value of −2.40 [39], and is highly water soluble. These chemical differences may contribute to the differences in reduction. The absolute composition of meglumine antimoniate may also contribute. Studies into structural characterization have focused on a 1:2 Sb(V) to NMG compositional ratio as the predominant form. However, studies by Frezard et al. have contested this composition, suggesting the drug is a mix of homoleptic complexes of varying metal to ligand ratios; 1:1, 1:2, 2:2, and 2:3 [40]. The rate at which each of these complexes undergoes reduction may differ as a consequence of their differing composition and structural chemistry, and so the reduction rate for 'meglumine antimoniate' is an average. In contrast, **S1** is a pure heteroleptic complex with an invariant composition of a 1: 3: 2 ratio of Sb(V), phenyl , and carboxylate.

Regardless, the rate of reduction is substantially slower than for the bismuth analogue **B1** which provides an insight into why bismuth complexes belonging to this family tend to display non-selective toxicity

towards the leishmanial and mammalian cells [23,24]. Studies into the interaction of antimoniates with trypanothione have concluded that it reduces Sb(V) at a much higher rate than GSH [15], and therefore it can be deduced that the reduction of the Bi(V) analogue would be even more facile. This reduction also occurs rapidly in the acidic conditions of the amastigote and macrophage [41]. This substantial difference between the reduction rate of Bi(V) *versus* Sb(V) organometallics by thiol rich peptides may help to us to understand the significant differences in their biological activity and selectivity in the treatment of Leishmaniasis.

4. Conclusions

This study has investigated the reduction of analogous organometallic complexes of Sb(V) and Bi(V) in a range of pH values with trypanothione, which is parasite specific, and its mammalian tripeptide analogue glutathione. In the clinically relevant pH range of 4–5, both $[BiPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ (**B1**) $[SbPh_3(O_2CCH_2(C_6H_4CH_3))_2]$ (**S1**), are rapidly reduced by trypanothione and glutathione. However, in the presence of glutathione at higher pH values differing reduction rates are observed. The highly oxidising bismuth complex **B1** underwent rapid reduction over a matter of a few minutes, whilst the reduction rate of the antimony complex **S1** was significantly slower, with complete degradation taking approximately 8 h. A half-life of $t = 54$ min was calculated for the logarithmic decay of complex **S1** into $SbPh_3$ and the parent *p*-tolylacetic acid. Formation of the $Sb(Gs)_3$ complex was absent for **S1**, however was observed to form from **B1**, $(Bi(GS)_3)$, providing some valuable insights into the chemical nature of the selective cytotoxicity observed for this class of complex. This rate of reduction was observed to be more rapid than that of the front-line anti-leishmanial drug meglumine antimoniate. This study has established a significant difference in the cellular chemistry between Bi(V) and Sb(V) triaryl carboxylates, which can be linked to their distinctly different biological activity and selectivity.

List of abbreviations

GSH	Glutathione
TSH	Trypanothione
GSSG	Oxidised glutathione
^{1}H NMR	Proton nuclear magnetic resonance
FT-IR	Fourier transform infrared
CLogP	Partition co-efficient
GS	Complexed glutathione

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2021.111470.

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