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Anti-Leishmanial activity of homo- and heteroleptic bismuth(III) carboxylates

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ABSTRACT

Bismuth(III) complexes of NSAIDs (Non-Steroidal Anti Inflammatory Drugs) and substituted benzoic acids (*o*-methoxybenzoic acid, *m*-methoxybenzoic acid, *o*-nitrobenzoic acid, 3,5-diacetamidobenzoic acid, and 5-[(*R/S*)-2,3-dihydroxypropyl carbamoyl]-2-pyridine carboxylic acid) have been synthesised and fully characterised. Two new *bis*-carboxylato bismuth complexes have been characterised by single crystal X-ray diffraction, namely [PhBi(*o*-$MeOC_6H_4CO_2$)$_2$(bipy)]·0.5EtOH (bipy = 2,2'-bipyridine) and [$PhBi(C_9H_{11}N_2O_3CO_2)_2(H_2O)$]·$6H_2O$. All compounds were tested against the parasite *Leishmania major* promastigotes for their anti-Leishmanial activity and were further assessed for their toxicity to mammalian cells. The NSAID free acids and their bismuth derivatives show negligible anti-Leishmanial activity at concentrations 1.95 to 250 μg/mL against the promastigotes of *L. major* whereas in the case of mammalian cells only bismuth complexes of naproxen and mefenamic acid have significant effect at concentration ≥ 250 μg/mL. The bismuth(III) complexes of substituted benzoic acids show significant anti-Leishmanial activity against the promastigotes of *L. major V121* at very low concentrations while their respective free carboxylic acids show no effective activity. However, the bismuth compounds inhibit the growth of the mammalian cells at all concentrations studied (1.95 to 500 μg/mL) following 48 h incubation. The comparatively low toxicity of $BiCl_3$ and $Bi(NO_3)_3$, suggests that overall toxicity of bismuth complexes towards the parasite is both ligand and metal dependent.

1. Introduction

Leishmaniasis is a group of diseases resulting from infection by the parasitic protozoa of the genus *Leishmania* and is transmitted to humans primarily through sandfly bites, though various domestic animals and humans can act as reservoirs for the parasite [1]. It is endemic in the developing world, affecting *ca* 12 million people in 88 countries with the World Health Organisation estimating two million new cases every year [2,3]. Most involve cutaneous (or mucocutaneous) Leishmaniasis (CL) resulting in simple ulceration through to the destruction of cutaneous and subcutaneous tissues. Visceral Leishmaniasis (VL), in contrast, results in organ damage and is generally fatal within two years if left untreated. Of the few drugs available to treat *Leishmania*, those based on Sb(V), sodium stibogluconate (Pentosam) and meglumine antimoniate (Glucantime), which were developed and first administered in the 1940s, remain the most important and cost effective [4]. However, there are substantial problems; the treatment regime for VL, requiring intramuscular injection daily for at least 28 days, has resulted in significant levels of non-compliance, and areas of drug resistance, particularly in northern Bihar, India, have emerged. The two main alternatives, Amphotericin B and Pentamidine, are expensive and do not overcome the need for parenteral administration or reduce the onset of severe side effects [2]. Miltefosine, an alkylphospholipid, is currently undergoing clinical trials and has been registered in several countries. It has the great benefit of being able to be taken orally, however, it is teratogenic in mammals and, despite good cure rates, exhibits a narrow therapeutic window [5,6].

Sb(III) complexes are too toxic for direct clinical administration, however, it is believed that the mechanism of action involves reduction of the Sb(V) species to Sb(III) inside the macrophage and parasite cell promoted by particular thiols; cysteine, cysteinyl-glycine and trypanothione (parasite specific) at pH 5 [4,7–10]. *In-vitro* reduction has been established using glutathione-S-transferase. It is suggested that redistribution of Sb(III) out of the parasite cell which is responsible for toxic side effects and that the control of Sb(III) may reduce or inhibit these unwanted effects.

Despite the close periodic relationship of antimony and bismuth, there are no reports in the literature of bismuth-based drugs being developed or evaluated as anti-Leishmanial agents. This most likely stems from perceived difficulties arising from the insolubility and instability of metal–organic bismuth compounds, and associated problems of preparation, storage and handling. However, given the reportedly anomalous lower toxicity of bismuth compounds [9,11–14] there is an opportunity to develop new drugs that display anti-parasitic activity while reducing unwanted side effects. Particularly enticing is the possibility of designing and delivering compounds directly in the more active reduced +3 oxidation state.

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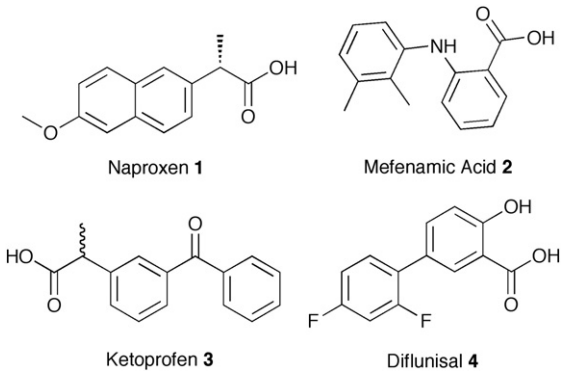

Fig. 1. Non-steroidal anti-inflammatory drugs (NSAIDs) (**1**–**4**) used in this study.

In this paper we now report the results of our initial study into the anti-Leishmanial activity of three related families of bismuth carboxylates: *tris*-carboxylato complexes derived from common non-steroidal anti-inflammatory drugs (NSAIDs) (**1**–**4**), shown in Fig. 1, and *bis*- and *tris*-substituted bismuth(III) carboxylates of the form $PhBiL_2$ and BiL_3 where the ligands (LH) are drawn from *o*-methoxybenzoic acid (**5**), *m*-methoxybenzoic acid (**6**), *o*-nitrobenzoic acid (**7**), 3,5-diacetamidobenzoic acid (**8**), and 5-[(*R*/*S*)-2,3-dihydroxypropylcarbamoyl]-2-pyridinecarboxylic acid (**9**), Fig. 2.

The *tris*-carboxylato bismuth(III) complexes derived from the NSAID acids **1**–**4** and those from the benzoic acids **5**–**7**, we have published previously. To expand the range of compounds for this study, we also synthesised and characterised the *tris*-carboxylato bismuth(III) derivative of 3,5-diacetamidobenzoic acid, and the heteroleptic *bis*-carboxylato complexes, $PhBiL_2$, derived from acids **5**–**7** and **9**. The synthetic and analytical details are described, alongside the crystal structure determination of two of the complexes; $[PhBi(o\text{-}MeOC_6H_4CO_2)_2(bipy)]$ (**19**) (bipy = 2,2′-bipyridine) prepared from **5**, and the water soluble complex $[PhBi(C_9H_{11}N_2O_3CO_2)_2(H_2O)]$ (**21**), prepared from **9**.

The toxicity of the compounds against *Leishmania major* promastigotes and human fibroblast cells is presented.

2. Results and discussion

2.1. Tris-carboxylato Bi(III) complexes

The synthesis and characterisation of *tris*-carboxylato bismuth(III) complexes derived from naproxen (**10**), mefenamic acid (**11**), ketoprofen (**12**) and diflunisal (**13**), *o*-methoxybenzoic acid (**14**), *m*-methoxybenzoic acid (**15**) and *o*-nitrobenzoic acid (**16**) are reported elsewhere [15,16]. The new homoleptic *tris*-substituted complex $[Bi\{(CH_3CONH)_2C_6H_3CO_2\}_3(H_2O)_3]$ (**17**) was synthesised from the reaction of 3,5-diacetamidobenzoic acid (**8**) with $BiPh_3$ in a 3:1 ratio under reflux in toluene. Both elemental and thermogravimetric analyses (TGA) indicated that three water molecules (5.5% wt/wt) are associated with the complex, and this is supported by the FT-IR spectrum which showed a strong absorbance due to OH stretching at 3285 cm^{-1}.

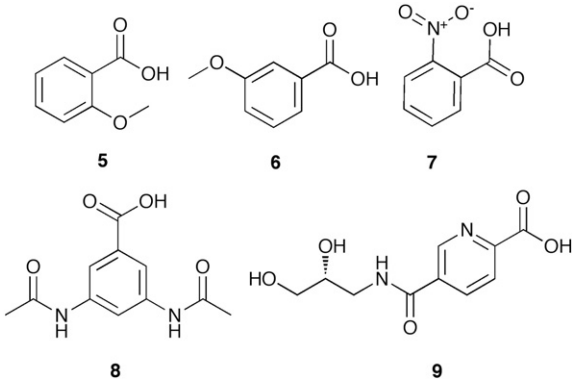

Fig. 2. Substituted benzoic acids (**5**–**9**) used in this study.

2.2. Bis-carboxylato phenylbismuth(III) complexes

Using a method similar to that which previously allowed us to synthesise and characterise $[PhBi(o\text{-}NO_2C_6H_4CO_2)_2]$ (**18**) [17], the heteroleptic *bis*-carboxylato complexes **19**, **20** and **21**, derived from *o*-methoxybenzoic acid (**5**), *m*-methoxybenzoic acid (**6**), and 5-[(*R*/*S*)-2,3-dihydroxypropyl carbamoyl]-2-pyridine carboxylic acid (**9**) were synthesised by the reaction of the substituted benzoic acid with $BiPh_3$ in a 2:1 ratio under reflux in either methanol or ethanol (Table 1).

Mirroring our observations on the behaviour of **18**, the reaction of **5** with $BiPh_3$ (2:1) in ethanol gives a mixture of products arising from the facile dismutation of the target compound $PhBiL_2$ to the homoleptic *tris*-substituted carboxylate, BiL_3 and the *mono*-substituted carboxylate, Ph_2BiL. Whitmire has recently reported some success in stabilising such *bis*-carboxylato bismuth complexes through the addition of 2,2′-bipyridyl (bipy) [18], and as such the reaction of *o*-methoxybenzoic acid was repeated with the addition of one equiv. of bipy. After a period of a few weeks, single crystals were obtained and identified by X-ray diffraction as $[PhBi(o\text{-}MeOC_6H_4CO_2)_2(bipy)]$ (**19**).

In contrast, the reaction of **6** with $BiPh_3$ (2:1) in ethanol gives the desired complex $[PhBi(m\text{-}MeOC_6H_4CO_2)_2(H_2O)_2]$ (**20**), as the major product, without the need to add bipy. This difference in stability most likely arises from the methoxy group in the *ortho* position in (**19**) donating electrons to both the aromatic ring and the carboxyl group, thereby increasing the lability of the carboxylate ligand, leading to a mixture of products. However, in the case of *m*-methoxybenzoic acid, the methoxy group donates electrons to the aromatic ring only and does not activate the carboxyl group, thereby increasing the relative stability of the *bis*-carboxylato complex to rearrangement.

The pyridine based amide-diol ligand **9** was designed and synthesised to increase both aqueous solubility and to improve overall complex stability through involvement of the pyridyl N in chelation to the bismuth centre. Our previous studies with picolinic acid showed that the *bis*-carboxylate was the thermodynamically favoured product even if the ratio of acid to $BiPh_3$ used in the reaction was 3:1 [16]. This was also found for the reaction of **9** with $BiPh_3$ which, on reflux in methanol (due to better solubility than in ethanol), produced $[PhBi(C_9H_{11}N_2O_3CO_2)_2(H_2O)]$ (**21**), from either a 2:1 or 3:1 reaction stoichiometry. The complex is soluble and stable in water, and crystals suitable for single crystal X-ray diffraction were obtained from water solution over a few weeks.

2.2.1. Characterisation of complexes 19, 20 and 21

The chemical composition of complexes **19**, **20** and **21** were confirmed through ^1H and ^{13}C NMR spectroscopy, FT-IR spectroscopy, electrospray ionization mass spectrometry (ESI-MS) and elemental analysis.

Table 1
Reaction of substituted benzoic acids with $BiPh_3$.

Acid	Reaction solvent	Complex	Appearance	M.pt (°C)
5	Ethanol (+1 equiv. bipy)	$[PhBi(o\text{-}MeOC_6H_4CO_2)_2(bipy)]$ **19**	Pale pink	129–130
6	Ethanol	$[PhBi(m\text{-}MeOC_6H_4CO_2)_2(H_2O)_2]$ **20**	Colourless	192–193
9	Methanol	$[PhBi(C_9H_{11}N_2O_3CO_2)_2(H_2O)]$ **21**	Colourless	> 300 (Dec.)