



# Synthesis and antimicrobial activity of a phenanthroline-isoniazid hybrid ligand and its Ag<sup>+</sup> and Mn<sup>2+</sup> complexes

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**Abstract** Hydrazide ligand, (*Z*)-*N'*-(6-oxo-1,10-phenanthroline-5(6H)-ylidene)isonicotinohydrazide, **1** forms from a 1:1 Schiff base condensation reaction between isoniazid (INH) and 1,10-phenanthroline-5,6-dione (phendione). Ag<sup>+</sup> and Mn<sup>2+</sup> complexes with 1:2 metal:ligand stoichiometry are prepared: [Ag(**1**)<sub>2</sub>]NO<sub>3</sub>, [Ag(**1**)<sub>2</sub>]BF<sub>4</sub> and [Mn(**1**)<sub>2</sub>](NO<sub>3</sub>)<sub>2</sub>. Polymeric {[Ag(**1**)(NO<sub>3</sub>)]<sub>n</sub>} has 1:1 stoichiometry and forms upon infusion of CH<sub>2</sub>Cl<sub>2</sub> into a DMSO solution of [Ag(**1**)<sub>2</sub>]NO<sub>3</sub>. {[Ag(**1**)(NO<sub>3</sub>)]<sub>n</sub>} was structurally characterized using X-ray crystallography. Metal-free **1** and its 1:2 complexes exhibit very good, broad-spectrum antimicrobial activity and are not excessively toxic to mammalian cells (A549 lineage).

**Keywords** 1,10-Phenanthroline · Metal complexes · Antibacterial activity · Antibiotic resistance · Anti-tuberculosis activity

## Introduction

Pathogenic bacteria, fungi, parasites and viruses can trigger sepsis, a condition whereby the body's response to infection induces tissue and organ dysfunction, leading to excessive morbidity and mortality. In both community and clinic settings septicemia is estimated to affect over 30 million people annually and causes up to 6 million fatalities (WHO factsheet 2018). The World Health Assembly (WHA) (Seventh WHA 2018) and the World Health Organization (WHO) (WHO factsheet 2018) both acknowledge that more investment is required for research into finding

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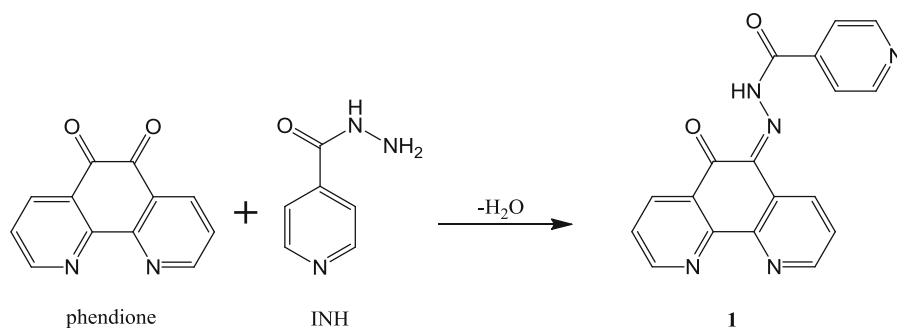
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new and cost-effective antimicrobials to overcome the major problem of drug resistance.

Since the 1950s, metal-free ligands and coordination complexes constituted by members of the 1,10-phenanthroline family of *N,N'*-chelating ligands have been shown to inhibit the growth of potentially harmful microorganisms (MacLeod 1952; Dwyer et al. 1957, 1969a, b; Shulman and Dwyer 1964; Butler et al. 1969, 1970; Cade et al. 1970; McCann et al. 2012a, b; Viganor et al. 2017; McCarron et al. 2018). Dwyer et al. (1969a, b) formulated and patented phenanthroline-containing therapeutics for topical application to humans, lower animals and plants in order to combat diseases induced by a wide variety of organisms. In 1970 in Melbourne, Australia, the results of two controlled clinical trials (Cade et al. 1970; Bulter et al. 1970) on patients suffering from topical infections due to fungi, bacteria and protozoa showed the highly beneficial effects of the topical application of formulations containing  $Mn^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$  complexes of 3,4,7,8-tetramethyl-1,10-phenanthroline. Additionally, the metal complexes were non-toxic and it was reported in the trial involving the  $Mn^{2+}$  complexes (Cade et al. 1970) that microbial resistance did not develop.

In the current paper, we outline the synthesis of a new Schiff base ligand, **1**, derived by reacting the anti-tuberculosis drug, isoniazid (INH), with 1,10-phenanthroline-5,6-dione (phendione) (Scheme 1).  $Ag^+$  and  $Mn^{2+}$  complexes of **1** were also prepared and one of the  $Ag^+$  species was structurally characterised. Also presented are the results of in vitro growth inhibition tests against bacterial (*Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungal (*Candida albicans*) pathogens. Additionally, the cytotoxicity of **1** and its complexes towards lung epithelial A549 cells was assessed and selectivity index (SI) values established.

**Scheme 1** Synthesis of ligand **1**



## Materials and methods

All reagents used in chemical syntheses were commercially available (Sigma-Aldrich) and were used as supplied. Phendione was synthesised using a literature procedure (Zheng et al. 2010). All experiments involving  $Ag^+$  compounds were conducted in the absence of light and samples were stored in the dark. NMR (Nuclear Magnetic Resonance) spectra were recorded on a Bruker Avance spectrometer operating at 500 MHz for  $^1H$  nucleus and 125 MHz for the  $^{13}C$  nucleus. The probe temperature was maintained at 25 °C. Electrospray ionization (ESI) High Resolution Mass Spectrometry (HRMS) analyses were collected on an Agilent-L 1200 Series coupled to a 6210 Agilent Time-of-Flight (TOF) equipped with both a positive and negative electrospray source.

Infrared spectra were recorded as KBr discs in the region 4000–400  $cm^{-1}$  on a Perkin-Elmer 100 series spectrometer. Elemental analyses (%CHN) were carried out using a FLASH EA 1112 Series Elemental Analyser with Eager 300 operating software. Magnetic moment measurements were taken at room temperature using a Johnson Matthey Magnetic Susceptibility Balance with  $Hg[Co(SCN)_4]$  being used as the calibration standard.

X-ray structural analysis for crystals of  $\{[Ag(\mathbf{1})(NO_3)]\}_n$  (Table 1) were performed on a Bruker APEX Duo CCD at 100(2) K with an Oxford Cobra cryostat, with the sample mounted on a MiTeGen microloop using Mo  $K\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). Bruker APEX (Bruker 2015) software was used to collect and reduce data and determine the space group. Absorption corrections were applied using SADABS (Bruker 2014–2015). Structures were solved with the XT structure solution program (Sheldrick 2015) using Intrinsic Phasing and refined with the XL refinement package (Sheldrick

**Table 1** Crystal data and structure refinement details for {[Ag(**1**)(NO<sub>3</sub>)]}<sub>n</sub>

Identification code	{[Ag( <b>1</b> )(NO <sub>3</sub> )]} <sub>n</sub>
Empirical formula	C <sub>18</sub> H <sub>11</sub> AgN <sub>6</sub> O <sub>5</sub>
Formula weight	499.20
Temperature (K)	100(2)
Wavelength (Å)	0.71073
Instrument	Bruker APEX DUO
Crystal system	Triclinic
Space group	P1
a (Å)	8.3219(15)
b (Å)	10.3830(18)
c (Å)	10.5249(19)
α (°)	73.092(2)
β (°)	80.063(3)
γ (°)	79.284(3)
Volume (Å <sup>3</sup> )	848.1(3)
Z	2
ρ (calc. Mg/m <sup>3</sup> )	1.955
μ (mm <sup>-1</sup> )	1.239
F(000)	496
Crystal size (mm <sup>3</sup> )	0.13 × 0.12 × 0.05
Theta range for data collection (°)	2.039 to 27.186
Reflections collected	11367
Independent reflections	3790 [R(int) = 0.0621]
Max. and min. transmission	0.7455 and 0.6472
Data/restraints/parameters	3790/1/275
Goodness-of-fit on F <sup>2</sup>	1.025
Final R indices [I > 2σ(I)]	R1 = 0.0419, wR2 = 0.0659
R indices (all data)	R1 = 0.0685, wR2 = 0.0719
Largest diff. peak and hole (e Å <sup>-3</sup> )	0.533 and - 0.875
CCDC No.	1907353

2008) using Least Squares minimisation in Olex2 (Dolomanov et al. 2009). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model with appropriately fixed isotropic thermal parameters. The amide hydrogen atom H9 was located and refined with restraints (DFIX).

#### Chemical synthesis

(*Z*)-*N'*-(6-oxo-1,10-phenanthroline-5(6*H*)-ylidene)isonicotinohydrazide (**1**)

1,10-Phenanthroline-5,6-dione (phendione) (0.210 g, 1.000 mmol) was added to solution of isonicotinic acid hydrazide (isoniazid, (INH)) (0.137 g,

1.000 mmol) in EtOH (25 cm<sup>3</sup>). *p*-Toluenesulfonic acid (10%, 0.02 g, 0.10 mmol) was added and the solution refluxed for 6 h. The resulting suspension was filtered whilst hot to remove a very small quantity of precipitated 1,10-phenanthroline-5,6-diol. The bright orange filtrate was allowed to stand in the dark overnight. Yellow needles of **1** were filtered off, washed with EtOH (3 × 10 cm<sup>3</sup>) and air-dried.

Yield: 0.263 g, 79%. MP: 210–211 °C.

Calculated (C<sub>18</sub>H<sub>11</sub>N<sub>5</sub>O<sub>2</sub>·0.5H<sub>2</sub>O): C, 63.90; H, 3.58; N, 20.70%. Found: C, 63.84; H, 3.39; N, 20.78%.

HRMS: calculated m/z for C<sub>18</sub>H<sub>11</sub>N<sub>5</sub>O<sub>2</sub>: (M + H)<sup>+</sup> 330.0986. Found: (M + H)<sup>+</sup> 330.0997.

IR (KBr, cm<sup>-1</sup>): 3424 [w. br], 3047.77 [w. br] 1723 [s. sh], 1635 [s. sh], 1576 [w. sh], 1557 [w. sh], 1509 [s.

br], 1445 [w], 1407 [m], 1223 [s. sh], 1180 [s. sh], 1064 [s. sh], 1011 [s. sh], 752 [m. sh], 686 [m. sh].

$^1\text{H NMR}$  ( $d^6$ -DMSO, 500 MHz):  $\delta$  15.13 (s, 1H, O–HN), 9.11 (dd,  $J = 4.5, 1.7$  Hz, 1H, PhenH), 8.94 (dd,  $J = 4.4, 1.6$  Hz, 2H, PyH), 8.90 (dd,  $J = 4.5, 1.7$  Hz, 1H, PhenH), 8.61 (dd,  $J = 7.9, 1.7$  Hz, 1H, PhenH), 8.50 (d,  $J = 7.9$  Hz, 1H, PhenH), 7.88 (dd,  $J = 4.4, 1.6$  Hz, 2H, PyH), 7.75 (dd,  $J = 7.9, 4.5$  Hz, 1H, PhenH), 7.67 (dd,  $J = 7.9, 4.5$  Hz, 1H, PhenH).

$^{13}\text{C NMR}$  ( $d^6$ -DMSO, 125 MHz):  $\delta$  181.9 (PhenC=O), 164.5 (C=O), 156.2 (PhenC), 152.7 (PhenC), 151.6 (PhenC), 151.4 (PyrC), 147.1 (PhenC), 139.4 (PyrC), 136.3 (PhenC), 135.1 (PhenC), 132.2 (PhenC), 128.6 (PhenC), 127.8 (PhenC), 125.6 (PhenC), 125.3 (PhenC), 122.0 (PyrC).

#### $[\text{Ag}(\mathbf{1})_2]\text{NO}_3$ and $[\text{Ag}(\mathbf{1})_2]\text{BF}_4$

A solution of  $\text{AgNO}_3$  or  $\text{AgBF}_4$  (0.085 g or 0.097 g, respectively, 0.500 mmol) in MeCN (20  $\text{cm}^3$ ) was added to a warm solution of **1** (0.329 g, 1.00 mmol) in MeCN (80  $\text{cm}^3$ ). The resulting suspension was refluxed for 3 h and filtered whilst hot to give an orange/yellow solid. The solid was washed with hot MeCN (3  $\times$  20  $\text{cm}^3$ ) and then with hot DCM (5  $\times$  20  $\text{cm}^3$ ), and dried under vacuum.

$[\text{Ag}(\mathbf{1})_2]\text{NO}_3 \cdot 2\text{H}_2\text{O}$  yield: 0.316 g, 76%.

Calculated  $[\text{Ag}(\text{C}_{18}\text{H}_{11}\text{N}_5\text{O}_2)_2]\text{NO}_3 \cdot 2\text{H}_2\text{O}$ : C, 50.01; H, 3.03; N, 17.82%. Found: C, 50.44; H, 2.79; N, 17.91%.

MS: calculated  $m/z$  for  $[\text{Ag}(\text{C}_{18}\text{H}_{11}\text{N}_5\text{O}_2)_2]^+$ : (M) $^+$  765.0876. Found: (M) $^+$  765.0873.

IR (KBr,  $\text{cm}^{-1}$ ): 3412 [w. br], 3078 [w. br], 1712 [s. sh], 1631 [s. sh], 1578 [w. sh], 1564 [w. sh], 1510 [s. br], 1455 [w], 1409 [m], 1340 [s. br], 1217 [s. sh], 1187 [s. sh], 1068 [s. sh], 1048 [m. sh], 1020 [s. sh], 844 [w. sh], 830 [w. sh], 749 [w. sh], 679 [m. sh], 633 [w. sh].

$^1\text{H NMR}$  ( $d^6$ -DMSO, 500 MHz):  $\delta$  15.15 (s, 2H, O–HN), 9.14 (dd,  $J = 4.8, 1.7$  Hz, 2H, PhenH), 8.97–8.93 (m, 6H, 2PhenH & 4PyH), 8.84 (dd,  $J = 8.0, 1.7$  Hz, 2H, PhenH), 8.73 (d,  $J = 8.0$  Hz, 2H, PhenH), 7.98 (dd,  $J = 8.0, 4.8$  Hz, 2H, PhenH), 7.93–7.90 (m, 6H, 2PhenH & 4PyH).

$^{13}\text{C NMR}$  ( $d^6$ -DMSO, 125 MHz):  $\delta$  180.9 (PhenC = O), 164.5 (C = O), 156.4 (PhenC), 152.2 (PhenC), 151.5 (PyrC), 149.6 (PhenC), 144.1 (PhenC), 139.3 (PyrC), 137.9 (PhenC), 134.7 (PhenC), 134.0 (PhenC), 130.1 (PhenC), 128.9

(PhenC), 127.3 (PhenC), 126.9 (PhenC), 122.1 (PyrC).

$[\text{Ag}(\mathbf{1})_2]\text{BF}_4$  Yield: 0.305 g, 72%.

Calculated  $[\text{Ag}(\text{C}_{18}\text{H}_{11}\text{N}_5\text{O}_2)_2]\text{BF}_4$ : C, 50.67; H, 2.60; N, 16.41%. Found: C, 50.02; H, 2.24; N, 16.06%.

MS: Calculated  $m/z$  for  $[\text{Ag}(\text{C}_{18}\text{H}_{11}\text{N}_5\text{O}_2)_2]^+$ : (M) $^+$  765.0876. Found: (M) $^+$  765.0908;

IR (KBr,  $\text{cm}^{-1}$ ): 3402 [w. br], 3101 [w. br], 1706 [s. sh], 1629 [m. sh], 1580 [w. sh], 1564 [w. sh], 1521 [s. br], 1458 [w], 1409 [s. sh], 1290 [w. sh], 1228 [m. sh], 1185 [m. sh], 1080–1030 [s. br  $\nu(\text{BF}_4)$ ], 1021 [s. sh], 846 [w. sh], 816 [w. sh], 747 [w. sh], 681 [m. sh], 631 [w. sh].

$^1\text{H NMR}$  ( $d^6$ -DMSO, 500 MHz):  $\delta$  15.14 (s, 2H, O–HN), 9.13 (dd,  $J = 4.8, 1.7$  Hz, 1H, 2PhenH), 8.95–8.92 (m, 6H, 2PhenH & 4PyH), 8.82 (dd,  $J = 8.0, 1.7$  Hz, 2H, PhenH), 8.70 (d,  $J = 8.0$  Hz, 2H, PhenH), 7.97 (dd,  $J = 8.0, 4.8$  Hz, 2H, PhenH), 7.94–7.83 (m, 6H, 2PhenH & 4PyH).

$^{13}\text{C NMR}$  ( $d^6$ -DMSO, 125 MHz):  $\delta$  180.5 (PhenC=O), 164.5 (C=O), 156.0 (PhenC), 151.8 (PhenC), 151.1 (PyrC), 149.2 (PhenC), 143.6 (PhenC), 138.8 (PyrC), 137.5 (PhenC), 134.2 (PhenCN), 133.5 (PhenC), 129.6 (PhenC), 128.3 (PhenC), 126.9 (PhenC), 126.5 (PhenC), 121.6 (PyrC).

Slow diffusion of DCM vapour into a saturated DMSO solution of  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3 \cdot 2\text{H}_2\text{O}$  over a period of 9 months resulted in the formation of yellow crystals of  $\{[\text{Ag}(\mathbf{1})(\text{NO}_3)]\}_n$  which were suitable for X-ray crystallographic analysis.

#### $[\text{Mn}(\mathbf{1})_2](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$

A solution of  $\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  (0.126 g, 0.500 mmol) in MeCN (20  $\text{cm}^3$ ) was added to a warm solution of **1** (0.329 g, 1.000 mmol) in MeCN (80  $\text{cm}^3$ ) and the bright yellow suspension refluxed for 3 h. The mixture was filtered whilst hot and the orange solid washed with hot MeCN (3  $\times$  20  $\text{cm}^3$ ) and then hot dichloromethane (5  $\times$  20  $\text{cm}^3$ ), and dried under vacuum.

Yield: 0.310 g, 70%.

Calculated  $[\text{Mn}(\text{C}_{18}\text{H}_{11}\text{N}_5\text{O}_2)_2](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ : C, 49.50; H, 3.00; N, 19.24%. Found: C, 49.67; H, 2.45; N, 19.02%.

IR (KBr,  $\text{cm}^{-1}$ ): 3422 [w. br], 3091 [w. br], 1719 [s. sh], 1642 [s. sh], 1575 [m. sh], 1520 [s. br], 1443 [s. br], 1421 [s. sh], 1385 [s. sh], 1303 [s. br], 1265 [m. sh], 1225 [s. sh], 1186 [m. sh], 1071 [m. sh], 1020 [s.

sh], 844 [w. sh], 819 [w. sh], 748 [m. sh], 682 [m. sh], 639 [w. sh].

$$\mu_{\text{eff}} = 6.2 \text{ B.M.}$$

## Biological testing

### Antibacterial activity

The antibacterial susceptibility profiles of Gram-positive *S. aureus* (ATCC 25923) and Gram-negative *P. aeruginosa* (ATCC 27853) were determined according to the protocol recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2012a, b) using a 96-well microtiter plate with Mueller–Hinton broth (MHB) containing different concentrations of each test compound (starting from a 20 mM solution in DMSO) and classical antibacterial agents (ciprofloxacin and ceftazidime). The lowest concentration of each compound that inhibited bacterial growth, as ascertained by the absence of visible turbidity in each well, was considered to be the MIC value.

### Anti-mycobacterial activity

Activities of the test compounds as well as isoniazid were assessed against *M. tuberculosis* H<sub>37</sub>Rv (ATCC 27294) using the microplate Alamar Blue Assay (Accumed International, Westlake, OH, USA) (Franzblau et al. 1998). This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods (Tortoli et al. 2002; Kontos et al. 2004). Mycobacteria were grown in Middlebrook 7H9 medium (Becton–Dickinson, Sparks, MD, USA) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) and 0.05% Tween 80 and on Middlebrook 7H10 solid agar medium (Becton–Dickinson) supplemented with 10% OADC. The MICs were determined as described previously (Franzblau et al. 1998). Briefly, all mycobacterial strains were prepared from scoops of solid culture and suspended in PBS plus Tween 80, to reach a concentration equivalent to McFarland Standard No. 1 (approximately  $3 \times 10^7$  colony-forming units (CFU)/mL) and then diluted in Middlebrook 7H9 medium supplemented with 10% OADC to about  $10^5$  CFU/mL. One hundred microliters of the bacterial suspensions were added to microtiter plates containing, in each well, twofold diluted drugs in 100  $\mu$ L of

Middlebrook 7H9 medium with 10% OADC. The volume of drug-free and mycobacteria-free wells was adjusted to 200  $\mu$ L and used as the control. The plates were incubated at 37 °C for 5 days. Twenty microliters of Alamar Blue dye and 12.5  $\mu$ L of 20% sterilized Tween 80 reagent were added. The plates were re-incubated at 37 °C for 24 h, and the colors of all wells were recorded. The MIC was defined as the lowest drug concentration that prevented a color change from blue to pink.

### Antifungal activity

Antifungal susceptibility testing was performed using *Candida albicans* (ATCC 36802) according to the standardized broth microdilution technique described by the CLSI (CLSI 2008) and was interpreted according to the CLSI published document (CLSI 2012a, b). The test compounds and the clinical antifungal drug, amphotericin B, were evaluated and the MICs were determined by visual inspection following incubation in a humid atmosphere at 35 °C for 48 h.

### Cytotoxicity assay

The effects of the test compounds on the viability of A549 cells (ATCC CCL-185, alveolar basal epithelial cell of human adenocarcinoma) was evaluated using the MTT assay (Mosmann 1983). First, the A549 cells were allowed to adhere in 96-well tissue culture plates, and then the cells were treated with increasing concentrations of the compounds and incubated up to 48 h at 37 °C in a 5% CO<sub>2</sub> atmosphere. Subsequently, the culture medium was discarded and the formation of formazan crystals was measured by adding MTT (5 mg/mL in PBS, 25  $\mu$ g/mL) and incubating the wells for an additional 3 h in the dark at 37 °C. The plates were centrifuged at 500 $\times$ g for 10 min, the supernatant removed, the pellet dissolved in 200  $\mu$ L of DMSO and the absorbance measured in an ELISA reader at 570 nm (SpectraMax Gemini 190, Molecular Devices, CA, USA). The 50% cytotoxicity inhibitory concentration (CC<sub>50</sub>) was determined by linear regression analysis.

## Results and discussion

### Chemical synthesis

The hydrazide ligand **1** was prepared in good yield using a Schiff base condensation reaction between INH and phendione under acidic conditions. Although our initial aim was to carry out a Schiff base reaction at both carbonyl groups of phendione (by treatment with two equivalents of INH) only the mono Schiff base hydrazide formed. Even in the presence of a large excess INH (up to four equivalents) the double Schiff base adduct was not isolated. In this context, our group has previously reported that not all intended double Schiff base reactions proceed as anticipated upon reaction of phendione with amines. For example, chiral, multi-ring products formed upon reaction of phendione with *N,N'*-bis(2-aminophenyl)ethylenediamine (Coyle et al. 2003) and diethylenetriamine (Lin et al. 2006). Also, combining phendione with urea produced bipyridine-glycoluril (Elmans et al. 1998) whilst the reaction with *L*-tyrosine methyl ester gave a novel phenanthroline-oxazine (McCann et al. 2013).

Whereas the IR spectrum of phendione contains a  $\nu(\text{C}=\text{O})$  band at  $1685\text{ cm}^{-1}$  the spectrum of **1** has the carbonyl stretching band at  $1723\text{ cm}^{-1}$  and an imine  $\text{C}=\text{N}$  stretch at  $1635\text{ cm}^{-1}$  (Supplementary Material). The  $^1\text{H NMR}$  spectrum of **1** ( $d^6$ -DMSO) (Supplementary material) showed an uncharacteristic downfield resonance for the amide H atom at 15.08 ppm, suggesting that H-bonding is taking place between it and the O atom of the ketone group on the backbone of the phendione moiety. H-bonding of this type has previously been documented for hydrazones of similar structure and it leads to keto-enol tautomerization (Benković et al. 2018; Cheon et al. 2002; Barbazán et al. 2010). The  $^1\text{H NMR}$  spectrum of **1** in  $d^4$ -methanol shows additional peaks in the region 7.5–9.1 ppm compared to that recorded in  $d^6$ -DMSO, indicating that the tautomerization process is more clearly observed in the former solvent. It is believed that it is the presence of the intramolecular H-bonding between the NH moiety of the hydrazone and ketone oxygen atom which causes the carbonyl carbon to be less electrophilic and thus decreases the susceptibility of this carbonyl group toward further Schiff base (imine) formation. Imine functionalities are well known to undergo hydrolysis back to starting materials (Yildiz 2016; Buss and Ponka 2003). Thus, the

stability of **1** towards hydrolysis was investigated by monitoring changes in the  $^1\text{H NMR}$  spectrum of a solution of the compound in  $d^6$ -DMSO upon addition of a few drops of  $\text{D}_2\text{O}$ . No spectral changes were observed over a period of 1 week at ambient temperature indicating the relative stability of the  $\text{C}=\text{N}$  moiety towards hydrolysis. The structure of **1** is expected to be essentially identical to that of the closely related hydrazones formed by reacting phendione with benzoic acid hydrazide and 2-hydroxybenzoic acid hydrazide (Barbazán et al. 2010).

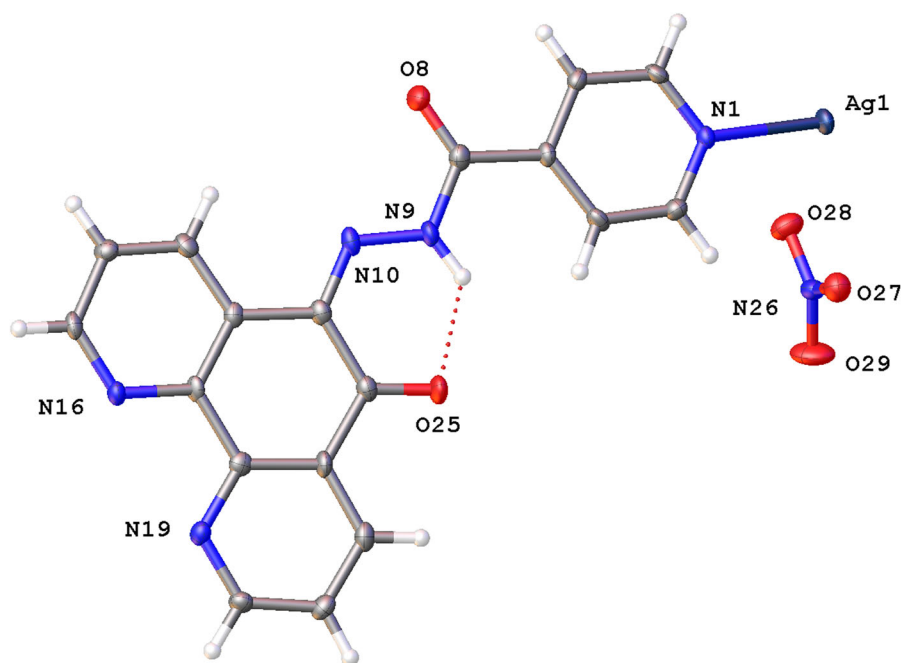
Metal complexes of **1** were synthesised by reacting simple  $\text{Ag}^+$  and  $\text{Mn}^{2+}$  tetrafluoroborate or nitrate salts with ligand **1** in a metal ion:ligand ratio of 1:2. The resulting complexes formulated as  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3$ ,  $[\text{Ag}(\mathbf{1})_2]\text{BF}_4$  and  $[\text{Mn}(\mathbf{1})_2](\text{NO}_3)_2$ . The IR spectra of the complexes contained bands associated with ligand **1** and the  $\text{NO}_3^-/\text{BF}_4^-$  anions (Supplementary material). The mass spectra of the  $\text{Ag}^+$  complexes showed peaks for the  $[\text{Ag}(\mathbf{1})_2]^+$  ion with characteristic isotopic patterns (Supplementary Material). Small shifts of the signals for the phenanthroline protons are seen when the  $^1\text{H NMR}$  spectrum ( $d^4$ -methanol) of the free ligand is compared to those of the  $\text{Ag}^+$  complexes. It is anticipated that in the core structure of the  $\text{Ag}^+$  and  $\text{Mn}^{2+}$  complexes the metal ion is chelated by both nitrogen atoms from the phendione moiety of each molecule of **1**, as has been shown previously in the cases of  $[\text{Ag}(\text{phendione})_2]\text{ClO}_4$  (McCann et al. 2004),  $[\text{Ag}(\text{phendione})(\text{CH}_3\text{CO}_2)]\cdot 3\text{H}_2\text{O}$  (Oneugbu et al. 2008) and  $[\text{Cu}(\text{phendione})_3](\text{ClO}_4)_2\cdot 2\text{H}_2\text{O}\cdot 2\text{MeCN}$  (Liu et al. 2000). Intermolecular coordination by the pyridine N atom of the INH moiety of **1** is also possible.

Interestingly, when the  $^1\text{H NMR}$  spectrum of  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3\cdot 2\text{H}_2\text{O}$  was recorded in  $d^6$ -DMSO new peaks appeared over a period of 3 days (Supplementary Material), the solution colour darkened visibly and precipitation was observed. These effects occurred both in the presence and in the absence of light but were not seen with the  $[\text{Ag}(\mathbf{1})_2]\text{BF}_4$  sample (NMR spectrum remained unchanged and the solution did not darken in the presence of light). Furthermore, slow diffusion of DCM vapour into a saturated DMSO solution of  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3\cdot 2\text{H}_2\text{O}$  over a prolonged period resulted in the formation of a new complex which X-ray crystallographic analysis revealed it to be polymeric and formulating with the 1:1 metal:ligand stoichiometry,  $\{[\text{Ag}(\mathbf{1})(\text{NO}_3)]\}_n$  (Figs. 1, 2, 3;

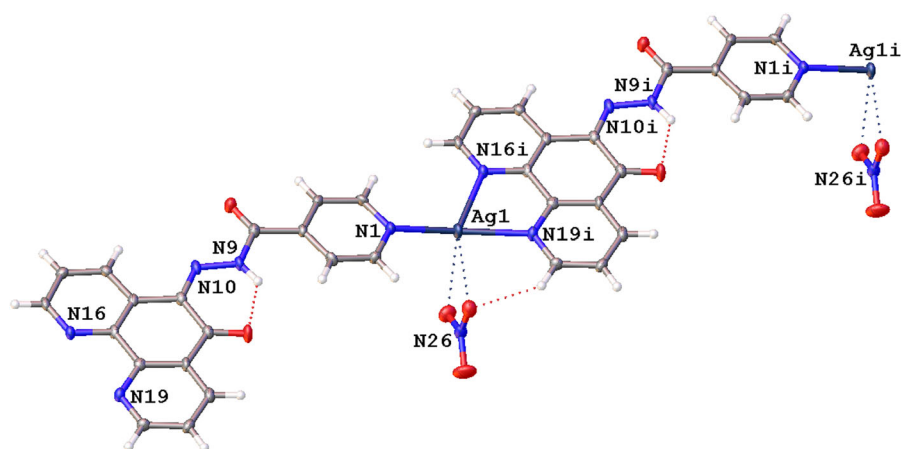
Table 2). In this complex, ligand **1** is seen to be ambidentate. Each  $\text{Ag}^+$  ion is bonded to three heterocyclic nitrogen atoms with the shortest Ag–N distance to the pyridine N atom of the INH moiety (Ag1–N1; 2.225(3) Å) and there are longer bonds to the *N,N'* chelating atoms of the phendione moiety of the adjacent ligand molecule (Ag1–N16<sup>i</sup> and Ag1–N19<sup>i</sup>; 2.434(3) and 2.314(3) Å, respectively; *i* = equivalent atom symmetry generation transformation  $x - 1, y + 1, z - 1$ ). The chelating phendione moiety creates the narrowest N–Ag–N angle (N19<sup>i</sup>–Ag1–N16<sup>i</sup>; 70.24(10)°), whilst N1–Ag1–N16<sup>i</sup> is

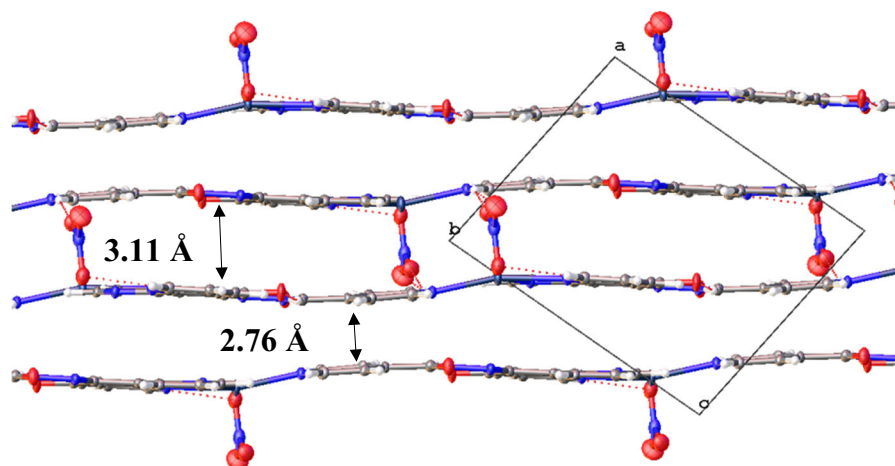
118.56(10)°. The N1–Ag1–N19<sup>i</sup> angle of 163.08(10)° is closest to being linear. The nitrate anion chelates to the central  $\text{Ag}^+$  ion (Ag–O28; 2.6468(28) and Ag–O27; 2.7822(27) Å), and it also serves to link the polymeric planes via extensive hydrogen bonding (Fig. 3). Finally, there is an intramolecular hydrogen bond within ligand **1** involving the hydrogen attached to N9 of the INH moiety and the carbonyl oxygen (O25) of the phendione moiety (Figs. 1, 2). The existence of such an intramolecular hydrogen bond within the metal-free ligand **1** was

**Fig. 1** Asymmetric unit of  $\{[\text{Ag}(\mathbf{1})(\text{NO}_3)]\}_n$  with selected atom numbering. Atomic displacement shown at 50% probability. Intramolecular hydrogen bonding shown as a dotted line



**Fig. 2** Symmetry generated (symmetry code used to generate equivalent atoms  $i = x - 1, y + 1, z - 1$ ) chain for  $\{[\text{Ag}(\mathbf{1})(\text{NO}_3)]\}_n$  with intramolecular hydrogen bonding showing the  $\text{Ag}^+$  coordination by the nitrate anion





**Fig. 3** Lamellar arrangement of  $\{[Ag(1)_2(NO_3)]_n\}$  viewed normal to the *b*-axis. The average distances between the planes described by the atoms in the ligand are ca. (a) 3.11 Å between

inferred earlier from the  $^1H$  NMR spectral studies of metal-free **1**.

#### Biological studies

The results of the biological screening studies for the metal-free ligand, **1**, the  $Ag^+$  and  $Mn^{2+}$  complexes of **1**, metal-free 1,10-phenanthroline, simple metal salts and some clinically used drugs are compiled in Tables 3 and 4.

#### Anti-*M. tuberculosis* activity

In terms of  $\mu M$  concentrations, the metal-free ligands, **1** and 1,10-phenanthroline, showed very similar activities against *M. tuberculosis* strain H<sub>37</sub>RV (Table 3), suggesting that the INH moiety of **1** is not playing a dominant role in inhibiting the growth of this bacterium. Furthermore, these two metal-free ligands were only marginally less effective than the prescribed drug, isoniazid. Whilst the simple metal salts,  $AgBF_4$  and  $Mn(NO_3)_2 \cdot 4H_2O$ , displayed only marginal ( $Ag^+$ ) to poor ( $Mn^{2+}$ ) activities, the metal complexes of **1**,  $[Ag(1)_2]BF_4$ ,  $[Ag(1)_2]NO_3 \cdot 2H_2O$  and  $[Mn(1)_2](-NO_3)_2 \cdot 2H_2O$ , were almost fourfold more potent than isoniazid. Interestingly, there was no real variation in activity across the three members of the group of metal complexes of **1**, suggesting that activity was independent of the type of chelated central metal ion ( $Ag^+$  or  $Mn^{2+}$ ). This is in stark contrast to the trend observed with the simple metal salts where the performance of

the two planes incorporating the nitrate anion via hydrogen bonding and (b) 2.76 Å separating these hydrogen bonded dimeric planes

**Table 2** Selected bond lengths [Å] and angles [°] for  $\{[Ag(1)(NO_3)]_n\}$

Ag1–N1	2.225(3)
Ag1–N16 <sup>i</sup>	2.434(3)
Ag1–N19 <sup>i</sup>	2.314(3)
O8–C7	1.212(4)
O25–C24	1.230(4)
N1–C2	1.336(4)
N1–C6	1.347(5)
N9–N10	1.347(4)
N9–C7	1.386(5)
N9–H9	0.899(10)
N1–Ag1–N16 <sup>i</sup>	118.56(10)
N1–Ag1–N19 <sup>i</sup>	163.08(10)
N19 <sup>i</sup> –Ag1–N16 <sup>i</sup>	70.24(10)

Symmetry transformations used to generate equivalent atoms:  $i = x - 1, y + 1, z - 1$

$AgBF_4$  was over 11-fold superior to  $Mn(NO_3)_2 \cdot 4H_2O$ . It was also evident that the potencies of the three metal complexes of **1** were significantly better than the combined activities of metal-free **1** and its respective simple metal salt, implying that dispensing the complex as a discrete entity was appreciably more beneficial than the additive effect of administering separate units of metal-free **1** along with the simple metal salt.

Encouragingly, preliminary tests involving three different rifampicin- and isoniazid-resistant *M. tuberculosis* strains (665/0514, SR Cult 84/17 and SR



**Table 3** Effects of test compounds on some clinically relevant microorganisms

Compounds	MIC ( $\mu\text{g/ml}$ ( $\mu\text{M}$ ))			
	<i>M. tuberculosis</i> H <sub>37</sub> Rv (ATCC 27294) <sup>a</sup>	<i>S. aureus</i> (ATCC 25923)	<i>P. aeruginosa</i> (ATCC 27853)	<i>C. albicans</i> (ATCC 36802)
<b>1</b>	5 (15.18)	16 (48.59)	128 (388.69)	4 (12.15)
[Ag( <b>1</b> ) <sub>2</sub> ]BF <sub>4</sub>	2.5 (2.93)	8 (9.38)	8 (9.38)	2 (2.34)
[Ag( <b>1</b> ) <sub>2</sub> ]NO <sub>3</sub> ·2H <sub>2</sub> O	2.5 (2.89)	16 (18.51)	8 (9.25)	2 (2.31)
[Mn( <b>1</b> ) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> ·2H <sub>2</sub> O	2.5 (2.99)	16 (19.10)	512 (611.29)	16 (19.10)
AgBF <sub>4</sub>	10 (51.36)	4 (20.54)	2 (10.27)	0.25 (1.29)
Mn(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	> 100 (> 558.82)	64 (355.14)	64 (355.14)	> 512 (> 2861.13)
1,10-Phenanthroline	2.5 (13.87)	> 512 (> 2861.13)	> 512 (> 2861.13)	0.25 (1.39)
Isoniazid	1.56 (11.38)	ND	ND	ND
Amphotericin B	ND	ND	ND	0.06 (0.06)
Ciprofloxacin	ND	4 (12.07)	4 (12.07)	ND
Ceftazidime	ND	16 (29.28)	4 (7.32)	ND

ND non-determined

<sup>a</sup>Rifampicin- and isoniazid-sensitive strain

**Table 4** Toxicity of test compounds on A549 epithelial cells and selectivity index (SI) values for the test microorganisms

Compounds	CC <sub>50</sub> , $\mu\text{M}$	Selectivity index (SI) <sup>a</sup> values			
		A549 epithelial cells	<i>M. tuberculosis</i> H <sub>37</sub> Rv (ATCC 27294)	<i>S. aureus</i> (ATCC 25923)	<i>P. aeruginosa</i> (ATCC 27853)
<b>1</b>	157.23	10.36	3.24	0.40	12.94
[Ag( <b>1</b> ) <sub>2</sub> ]BF <sub>4</sub>	44.11	15.05	4.70	4.70	18.85
[Ag( <b>1</b> ) <sub>2</sub> ]NO <sub>3</sub> ·2H <sub>2</sub> O	48.47	16.76	2.62	5.24	21.01
[Mn( <b>1</b> ) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> ·2H <sub>2</sub> O	56.67	18.95	2.97	0.09	2.97
AgBF <sub>4</sub>	95.53	1.86	4.65	9.30	74.05
1,10-Phenanthroline	2422.29	174.64	6.82	6.82	1742.65
Isoniazid	3500.10	307.57	ND	ND	ND
Amphotericin B	8.59	ND	ND	ND	143.17

ND non-determined

<sup>a</sup>SI = CC<sub>50</sub> value/MIC value

2571/0215) have revealed that **1** and its Ag<sup>+</sup> and Mn<sup>2+</sup> complexes greatly impede the growth of these highly resilient bacteria as similarly detected against the sensitive strain, H<sub>37</sub>RV (data not shown).

#### Anti-*S. aureus* and *P. aeruginosa* activity

Metal-free **1** and its metal complexes were up to sixfold less active against Gram-positive *S. aureus* in comparison to *M. tuberculosis* (Table 3). In contrast to the observation of the similar activities of metal-free **1** and metal-free 1,10-phenanthroline towards *M.*

*tuberculosis*, the latter ligand was ca. 60-fold less potent than **1** against *S. aureus*, implying that the INH moiety of **1** is exerting a very influential and positive effect on its activity against this bacterium.

*S. aureus* was more sensitive to AgBF<sub>4</sub> and Mn(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O than *M. tuberculosis*, with the Ag<sup>+</sup> simple salt being the most effective. [Ag(**1**)<sub>2</sub>]BF<sub>4</sub>, [Ag(**1**)<sub>2</sub>]NO<sub>3</sub>·2H<sub>2</sub>O and [Mn(**1**)<sub>2</sub>](NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O had activities similar to those of the clinical drugs, ciprofloxacin and ceftazidime, the remaining compounds being much less effective.

Metal-free **1** and  $[\text{Mn}(\mathbf{1})_2](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$  were notably much less effective towards Gram-negative *P. aeruginosa* than against *M. tuberculosis* and Gram-positive *S. aureus*. Only  $[\text{Ag}(\mathbf{1})_2]\text{BF}_4$  and  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3 \cdot 2\text{H}_2\text{O}$  had efficiencies approaching those of ciprofloxacin and ceftazidime towards *P. aeruginosa*.

#### Anti-*C. albicans* activity

With the exception of the  $\text{Mn}^{2+}$  simple salt,  $\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , all of the test compounds were very active against the fungus, *C. albicans*. Although the compounds were much less effective than the clinical drug, amphotericin B (MIC 0.06  $\mu\text{M}$ ), the latter polyene is reputed to have severe nephrotoxicity associated with it which often prompts a discontinuation of therapy despite a life-threatening, systemic fungal infection (Sabra and Branch 1990).

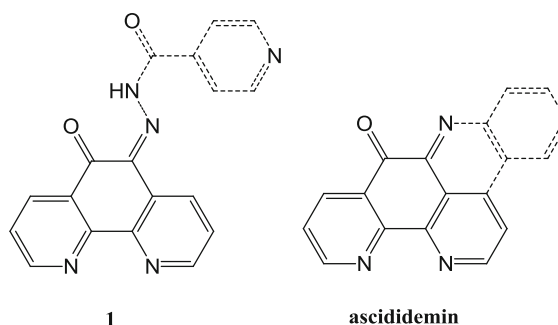
#### Toxicity versus A549 cell line

Mammalian A549 cells were the least sensitive to metal-free isoniazid, followed by metal-free 1,10-phenanthroline (Table 4). In comparison, metal-free **1**, which is a molecule whose framework is essentially a fusion of isoniazid with 1,10-phenanthroline, was much less well tolerated by the A549 cells. The  $\text{Ag}^+$  and  $\text{Mn}^{2+}$  complexes of **1** were ca. threefold more toxic towards these cells than metal-free **1**. Amphotericin B had the most detrimental effect on the A549 cells.

With respect to *M. tuberculosis* H<sub>37</sub>RV, isoniazid and metal-free 1,10-phenanthroline had the largest calculated Selectivity Index (SI) values (Table 4). Although metal-free **1** and its metal complexes had considerably lower SI values they still fell within the acceptable range ( $> 10$ ) (Nguta et al. 2015).

With the exception of  $\text{AgBF}_4$ , the SI values for both *S. aureus* and *P. aeruginosa* were very much lower than those found for *M. tuberculosis* H<sub>37</sub>RV across the range of test compounds (no value  $> 10$ ). Against the fungus, *C. albicans*, metal-free 1,10-phenanthroline returned an extremely large SI value (1742) and this was followed by amphotericin B (143) and the simple metal salt,  $\text{AgBF}_4$  (74). Metal-free **1** and its two  $\text{Ag}^+$  complexes had SI values in the range 13–21, with the  $\text{Mn}^{2+}$  complex giving the lowest value (ca. 3).

Finally, it is noteworthy that the core structure of ligand **1** is identical to that of the naturally occurring



**Fig. 4** Comparison of the core structures (shown in solid bonds) of **1** and ascididemin

marine alkaloid, ascididemin (Fig. 4), which is known to display potent antimicrobial (Bhakuni and Rawat 2010; Lindsay et al. 1995) and antitumor (Delfourne and Bastide 2003; Matsumoto et al. 2003) activity in its metal-free form. It is interesting that metal-free **1** and ascididemin (Lindsay et al. 1995) were both relatively inactive against Gram-negative *P. aeruginosa*. Contrastingly, whereas the current synthetic compounds are somewhat non-toxic towards human lung epithelium A549 cells ( $\text{CC}_{50} = 157.23 \mu\text{M}$ ) these cells are quite intolerant of the pyridoacridine alkaloid ( $\text{IC}_{50} = 0.02 \mu\text{M}$ ) Delfourne and Bastide 2003).

#### Conclusions

Ligand **1** and its  $\text{Ag}^+$  and  $\text{Mn}^{2+}$  complexes are readily obtained with robust yields from relatively cheap starting materials. Ligand **1** is not susceptible to immediate hydrolysis in presence of  $\text{H}_2\text{O}$  over a period of 7 days and the stability of the  $\text{Ag}^+$  complexes varies with the nature of the counterion. The  $[\text{Ag}(\mathbf{1})_2]\text{NO}_3$  complex shows signs of decomposition with 12 h in a DMSO solution, whereas the  $[\text{Ag}(\mathbf{1})_2]\text{BF}_4$  complex does not display instability in similar conditions when tested over 3 days. The activity displayed by ligand **1** is comparable to **INH** towards *M. tuberculosis* H<sub>37</sub>RV with its  $\text{Ag}^+$  and  $\text{Mn}^{2+}$  complexes being four times more active. These compounds are more selective towards *M. tuberculosis* H<sub>37</sub>RV over mammalian A549 cells and display SI values  $> 10$ . In addition, these compounds have very good, broad-spectrum antimicrobial activity.

## Supplementary information

Crystallographic data, CCDC 1907353 can be obtained free of charge from the Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif). Electronic supplementary material available.

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