Silver derivatives of multi-donor heterocyclic thioamides as antimicrobial/anticancer agents: unusual bio-activity against methicillin resistant S. aureus, S. epidermidis, and E. faecalis and human bone cancer MG63 cell line†

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The basic aim of this study pertains to the synthesis of silver nitrate complexes and the study of their antimicrobial and anticancer bio-activity. A series of new silver(I) derivatives of N-substituted-imidazolidine-2-thiones (L-NR, R = Et, Prn, Bu*, Ph), purine-6-thione (purSH2), 2-thiouracil (tucH2), pyrimidine-2-thione (pymSH) and pyridine-2-thione (pySH) of composition [Ag(S-L-NR)(PPh3)2(ONO2)] (R = Et (1), Prn (2), Bu* (3), Ph (4)), [Ag2(N,S-purSH2)2(u-dppm)2(NO3)2·2H2O (5) (dppm = Ph2P·CH2P·Ph2)],[Ag(L)(PPh3)]2(ONO2) (L = N,S-purSH2 (6), S-tucH2 (7)). [Ag(N,S-pymSH)(PPh3)2]2[CH3OH] (8), and [Ag(N,S-pySH)(PPh3)2]$2\text{-}H2O(9)$ have been synthesized and structurally characterized. These new and some previously reported complexes ([Ag2(L-NH)4(PPh3)2](NO3)2 (10), [Ag(L-NMe)2(PPh3)](NO3) (11), and [Ag(S-bzimS)2(PPh3)2](OAc) (12), L-NH = 1,3-imidazolidine-2-thione, L-NMe = 1-methyl-3-imidazolidine-2-thione and bzimS$2$ = benzimidazole-2-thione) have shown moderate to high anti-microbial activity against Gram positive bacteria, namely methicillin resistant Staphylococcus aureus (MRSA) and Staphylococcus aureus (MTCC 740), and Gram negative bacteria, namely Staphylococcus epidermidis (MTCC 435), Enterococcus faecalis (MTCC 439), Shigella flexneri (MTCC 1457) and a yeast Candida albicans (MTCC 22). These complexes have also been found to be bio-safe as studied using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The anti-tumor study of silver complexes against human osteosarcoma cell line (MG63) has shown IC50 values in the range of 6–33 μM.

**Introduction**

Silver is an important metal known since ancient times for its anti-septic and antibacterial properties. Silver compounds are used to control bacterial growth in a variety of medical applications. For example, silver nitrate was introduced in therapeutic applications in 1884 for the prevention of ophthalmia neonatorum, and in eye drops against eye infections. Further, silver(I) sulfadiazine is used to treat severe burns to protect them from bacterial infection and catalyzes skin renewal, while a silver sulfadiazine-chlorhexidine mixture is used against catheter infections in vivo. Silver is even present in the human body (absorbed through the lungs, gastrointestinal tract, mucus membranes and skin) and any excess amount is eliminated from the body through the urine and faeces. Polymeric silver compounds of ethanediyl bis(isonicotinate) have also been used in coatings of implant materials for killing bacterial infections while remaining biocompatible for cell growth. In view of the above uses, the investigation of silver(I) chemistry becomes exceptionally appealing, which is bolstered by the ability of this metal to form complexes of variable geometry and nuclearity. Literature reports reveal that silver(I) complexes of sulfur containing ligands have found application in medicines, analytical chemistry and polymer industry.
pyridine-2-thione, benzoxazoline-2-thione, thiazolidine-2-thione, and benz-imidazoline-2-thione, using different anions (NO$_3^-$, ClO$_4^-$, F, F$_2$SO$_3^-$, NCS$^-$, SO$_3^-$, BF$_4^-$) as well as halides has been reported.\textsuperscript{13-44} Among them several silver complexes have found use as anti-microbial, anti-tumor,\textsuperscript{17,18,36,19} anti-inflammatory\textsuperscript{18,23} and anti-malarial agents.\textsuperscript{29} In regard to the antimicrobial activity, complexes of silver(i) salts with the thio-ligands, namely, imidazolidine-2-thione,\textsuperscript{30,36} thiazoline-2-thione,\textsuperscript{30} benzo-thiazoline-2-thione,\textsuperscript{30} N-methyl-imidazoline-2-thione,\textsuperscript{30} pyrimidine-2-thiones,\textsuperscript{23} 4,6-diamino-5-hydroxyprymidine-2-thione,\textsuperscript{35} pyridine-2-thione,\textsuperscript{30} diazinane-2-thione,\textsuperscript{23} and 2-mercapto-nicotinic acid\textsuperscript{23} have shown activity against Escherichia coli,\textsuperscript{23} Pseudomonas aeruginosa,\textsuperscript{30,36} Bacillus subtilis,\textsuperscript{23,37} Bacillus cereus,\textsuperscript{23} Staphylococcus aureus,\textsuperscript{23,35,37} Pseudomonas aeruginosa,\textsuperscript{36} Penicillium citrinum,\textsuperscript{30,37} Rhizopus stolonifer,\textsuperscript{37} Aureobasidium pullulans,\textsuperscript{37} Fusarium moniliforme,\textsuperscript{34} Cladosporium sphaerospermum,\textsuperscript{36} and yeasts {Candida albicans,}\textsuperscript{26,32,34} Candida tropicalis,\textsuperscript{26,32,34} and C. stellatoideas.\textsuperscript{26} It is pointed out here that the investigations merely report antimicrobial activity without any mention of their biosafety.\textsuperscript{23,30,35,37,39,41,42} The anti-cancer/tumor activity of complexes of silver(i) with pyrimidine-2-thiones,\textsuperscript{36} tetrahydropyrimidine-2-thione,\textsuperscript{17} pyridine-2-thiones,\textsuperscript{36} thiazolidine-2-thione,\textsuperscript{39} 5-chloro-2-mercapto-benzothiazole,\textsuperscript{18} and 2-mercaptonicotinic acid,\textsuperscript{18} pertains to in vitro cytotoxic activity against murine leukemia (L1210),\textsuperscript{17,16} human T-lymphocyte (Molt4/C8 and CEM) cells,\textsuperscript{17,36} breast cancer cell lines (MDA-MB-231, MCF-7),\textsuperscript{39} colon cancer cell line (HT-29),\textsuperscript{39} leiomyosarcoma cancer cells (LMS),\textsuperscript{18} and breast cancer cell line (MDA-MB-231),\textsuperscript{18} some of which have shown activity even higher than that of the anti-cancer drug cisplatin.

From our laboratory, coordination chemistry of coinage metals (Cu, Ag) with heterocyclic-2-thiones has been investigated which showed interesting chemical activity such as rupture of C-S bond\textsuperscript{25-40} and formation of a variety of coordination complexes.\textsuperscript{59-57} As a part of our interest to pursue bioactivity of metal-heterocyclic-2-thione complexes, we recently reported synthesis and antimicrobial activity of several copper(i)/silver(i) halide complexes of imidazolidine-2-thiones (L-NR, R = H, Me, Et, Pr$_n^+$, Bu$_n^+$, Ph) with or without triphenylphosphate (PPh$_3$) as a co-ligand.\textsuperscript{44,58,59} These complexes showed moderate to high activity against various bacteria, Staphylococcus aureus, methicillin resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhimurium, Shigella flexneri, Escherichia coli, Candida albicans and Candida tropicalis, but only a few of them were biosafe.\textsuperscript{44,58,59}

Keeping in view the high profile medicinal activity of silver(i) compounds with nitrate as anion as well as in continuation of our interest in this area,\textsuperscript{44,58,59} in this investigation, a series of silver derivatives of thio-ligands, N-substituted-imidazoline-2-thiones, (L-NR, R = Et, Pr$_n^+$, Bu$_n^+$, Ph), purine-6-thione (purSH$_2$), 2-thiouracil (tucH$_2$), pyrimidine-2-thione (pymSH), and pyridine-2-thione (pySH) (Chart 1) are synthesized and structurally characterized. These complexes (1–9) as well as a few previously reported ones, namely, [Ag$_3$(L-NH$_2$(PPh$_3$)$_2$]$\cdot$NO$_3$]$_2$, [Ag(L-NMe$_2$(PPh$_3$))$\cdot$NO$_3$]$_2$, and [Ag(bzimSH)$_2$(PPh$_3$)$_2$]$\cdot$OAc$_2$] (10–12) [L-NH = 1,3-imidazolidine-2-thione; L-NMe = 1-methyl-3-imidazolidine-2-thione and bzimSH$_2$ = benz-imidazoline-2-thione]\textsuperscript{44} have been evaluated for their in vitro antimicrobial activity against various microorganisms (Gram positive bacteria: Staphylococcus aureus (MTCC 740), methicillin resistant Staphylococcus aureus (MRSA) Staphylococcus epidermidis (MTCC 435), Enterococcus faecalis (MTCC 439), Gram negative bacteria Shigella flexneri (MTCC 1457), Escherichia coli (MTCC 119) and a yeast Candida albicans (MTCC 227). Apart from antimicrobial studies, as an extension of bioactivity, some studies are explored in the use of complexes for their anticancer activity against MG63 – a bone cancer cell line.

**Results and discussion**

**Synthesis and IR NMR spectroscopy**

In this section, comments regarding synthesis of complexes (Chart 2) and IR spectral studies are described. In a typical
reaction, to the solution of silver(I) nitrate in methanol was added two m mole of solid L-NEt ligand followed by stirring for 1 h, and to the clear solution was added two m mole of solid PPh3, and the solution was further stirred for 15 minutes. The colorless contents were transferred into a culture tube for crystallization and after about one week crystalline product was obtained whose analytical data supported the composition as \([\text{Ag}(\text{S-L-NEt})(\text{PPh3})_2(\text{ONO}_2)]\). Similarly, other complexes, namely, \([\text{Ag}(\text{S-L-NR})(\text{PPh3})_2(\text{ONO}_2)]\) \((R = \text{Pr}^\text{r}, 2; \text{Bu}^\text{r}, 3; \text{Ph}, 4)\) were synthesized and bonding established using X-ray crystallography (vide infra). The crystalline pale yellow complex, \([\text{Ag}_2(\text{N,S-purSH}_2)(\text{dppm})_2](\text{ONO}_2)\) \(5\), was obtained using same procedure from a reaction of silver nitrate with purine-6-thione (purSH2) and bis(diphenylphosphino)methane (dppm). Complexes \([\text{Ag}(\text{N,S-purSH}_2)(\text{PPh3})_2](\text{ONO}_2)\) \(6\), \([\text{Ag}(\text{S-tucH}_2)(\text{PPh3})_2](\text{ONO}_2)\) \(7\), \([\text{Ag}(\text{L})(\text{PPh3})_2] \(L = \text{N,S-pymS}, 8; \text{N,S-pys} 9\) \) were prepared from the reactions of silver(I) nitrate with two m mole of solid PPh3 in methanol and the respective thio-ligands, purSH2, tucH2, pymSH and pysH with the only difference in case of the latter two complexes, where in Et3N Lewis base was added.

The significant IR bands of complexes 1–9 are given in Table 1. The free thio-ligand, L-NEt shows NH proton signal at 6.39 ppm which shifted to low field at 9.25 ppm in its complex 1 and this shift suggests that the thio-ligand is coordinating to the silver(I) ion as a neutral ligand. Similarly, low/high energy shift of \(v(\text{N–H})\) band of other thio-ligands, L-NPr\(^{\text{r}}\), L-NBu\(^{\text{r}}\), L-Ph, purSH2, tucH2, pymSH and pysH with the only difference in case of the latter two complexes, where in Et3N Lewis base was added.

**NMR spectroscopy**

Proton NMR signals of coordination compounds 1–9, free thioligands and co-ligands (PPh3, dppm) are given in Table 2. The thio-ligand, L-NEt, shows NH proton signal at 6.39 ppm which significantly shifted to low field at 9.25 ppm in its complex 1 and this shift suggests that the thio-ligand is coordinating to the silver(I) ion through its S-donor atom, as a neutral ligand. The ring and side chain protons, C\(^1\)H, C\(^2\)H and N–CH\(_2\) of thioligand L-NEt showed an unresolved multiplet at 3.59(m; 6H) respectively, whereas other two bands (I and II) remained less influenced (major contribution from \(\delta(\text{N–H}) + \delta(\text{C–H}) + \nu(\text{C–N}) + \nu(\text{C–S})\)) of the former two complexes, where in Et3N Lewis base was added.

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ligands were coordinating as neutral ligands. The uncoordinated PPh3 showed a multiplet at 7.32 ppm due to o-, m-, and p-H hydrogens of phenyl rings and in complexes 1–4, 6–9, this multiplet separated into ortho-hydrogen and meta-/para-hydrogen signals. For example, in complex 1 new multiplets appeared at low field, 7.52 ppm (m, o-H) and 7.40 ppm (m, m- and p-H). Other complexes 2–4, 6–9 showed similar trends. In dppm complex 5, the –CH2– moiety shows a signal at 3.79 ppm which is low field relative to the –CH2– moiety of free dppm, at 2.18 ppm. The phenyl ring protons of dppm are resolved into ortho, meta and para hydrogen signals in its complex 5. Thus proton NMR shows coordination by PPh3 and dppm in their complexes.

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Similarly, in complexes 3 and 6, the C² carbon signal has undergone upfield shift to 180.0 and 163.6 ppm, relative to the free ligands (L-Bu³, purSH₂ = 183.4, 171.4 respectively). Complexes of purine-6-thione (5), 2-thiouracil (7) pyrimidine-2-thione (8) showed small lowfield shifts of C² carbon (171.6 6, 163.6 7, 183.2 8 ppm), relative to the free ligands (purSH₂, tucH₂, pynSH = 171.4, 161.54, 181.9 ppm respectively). Further, the ring C⁴, C⁵ and side chain N-R carbons in complexes 1 and 3 as well as the C⁴⁺ carbons of complex 8 showed only minor variations as compared to the free thioligands. In complex 5, the ring C² and C⁴⁺ carbon signals (145.9, 133.3 ppm) of purSH₂ have undergone up-field shift in complex 5 relative to the free ligand (152, 145 ppm), due to the deshielding of these carbons. Similar trends are seen in complex 7 in comparison to the free ligand tucH₂. The ipso, ortho, meta and para carbon signals of coordinated PPh₃ in complexes 1, 3 and 6-8, undergo variations as follows: (i) ipso carbons have shown upfield shifts in all the complexes, ortho carbons have shown upfield shifts in complexes 1, 3, 6, 7 and lowfield shift in complex 8, meta- and para-carbons showed only minor variations in either direction in all the complexes. Whereas, complex 5, having dpmm as a co-ligand, ipso-, and ortho- carbons showed upfield shift and meta-, para-carbons of P-Ph moiety along with carbon atoms of P-CH₂-P group have

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### Table 2 ¹H NMR spectral data (δ in ppm) of complexes 1–9 and free ligands

| Complex/free thio-ligand | -NH protons | Ring protons of thio-ligand and co-ligands (PPh₃/dppm) |
|--------------------------|-------------|---------------------------------------------------|
| [Ag(S-L-NEt)(PPh₃)]₂[O(NO₂)] | 9.25 (s, 1H, NH) | 3.72 (m, 4H, C²H₄/C²H₃) 3.59 (q, 2H, N-CH₂) 1.21 (t, 3H, CH₃) 7.51 (m, 12H, o-H) 7.40 (m, 18H, m-H, p-H, PPh₃) |
| [Ag(S-L-NPr)₃(PPh₃)]₂[O(NO₂)] | 7.87 (s, 1H, NH) | 3.58 (q, 2H, N-CH₂) 3.39 (t, 2H, N-CH₂) 2.39 (t, 2H, CH₂) 1.51 (m, 2H, CH₂) 0.85 (t, 3H, CH₃) 7.32 (m, 12H, o-H) 7.23 (m, 18H, m-H, p-H, PPh₃) |
| [Ag(S-L-NBu)₃(PPh₃)]₂[O(NO₂)] | 8.40 (s; 1H, N⁵H) | 3.62 (dt, 2H, C²H₂) 3.62 (dt, 2H, N-CH₂) 3.50 (t, 2H, CH₂) 1.54 (quint, 2H, CH₂) 1.35 (m, 2H, CH₂) 0.95 (t, 3H, CH₃) 7.39 (m, 12H, o-H) 7.33 (m, 18H, m-H, p-H, PPh₃) |
| [Ag(S-L-NPh)(PPh₃)]₂[O(NO₂)] | 9.40 (s, 1H, NH) | 4.18 (t, 2H, C²H₂) 3.86 (t, 2H, C²H₂) 7.36 (m, 21H, o-H, P-P, PPh₃+L-NPh) 7.26 (m, 14H, m-H, P-P, PPh₃+L-NPh) |
| [Ag₂(N,S-purSH₂)₃(μ-dppm)] (NO₃) | 13.78 (s, 2H, N⁵⁹H) | 8.29 (s, 1H, C²H) 7.73 (s, 1H, C²H) 3.79 (s, 2H, P-CH₂-P, dppm) 7.36 (d, 8H, o-H) 7.20 (t, 4H, p-H) 7.06 (t, 8H, m-H, dppm) |
| [Ag₂(N,S-purSH₂)₃(NO₂)] | 13.78 (s, 2H, N⁵⁹H) | 8.40 (s, 1H, C²H) 8.33 (s, 1H, C²H) 7.41 (m, 12H, o-H) 7.27 (m, 18H, m-H, p-H, PPh₃) |
| [Ag₂(N,S-tucH₂)₃(NO₂)] | 8.28 (s; 2H, N⁴¹H) | 7.13 (d; 1H, C²H) 5.55 (d; 1H, C²H) 7.37 (m, 12H, o-H) 7.25 (m, 18H, m-H, p-H, PPh₃) |
| [Ag₂(N,S-pymS)₃(PPh₃)] | 8.28 (s; 2H, N⁴¹H) | 7.85 (d; 2H, C²H) 6.50 (t; 1H, C²H) 7.34 (m, 12H, o-H) 7.24 (m, 18H, m-H, p-H, PPh₃) |
| [Ag₂(N,S-pyS)₃(PPh₃)] | 8.28 (s; 2H, N⁴¹H) | 7.39 (t; 1H, C²H) 7.24 (m, 1H, C²H) 6.81 (t; 1H, C²H) 7.57 (m, 13H, C²H +o-H, PPh₃) 7.51 (m, 18H, m-H, p-H, PPh₃) |

| L-NEt⁶ | 6.39 (s, 1H, NH) | 3.59 (m, 6H, C²H₂/C²H₃/N-CH₂) 1.14 (s, 3H, CH₃) |
| L-NPr⁶ | 5.91 (s, 1H, NH) | 3.71 (t, 2H, C²H₂) 3.58 (m, 2H, N-CH₂) 3.46 (t, 2H, CH₂) 1.65 (m, 2H, CH₂) 0.97 (t, 3H, CH₃) |
| L-NBu⁶ | 6.02 (s, 1H, NH) | 3.64 (m, 2H, C²H₂) 3.53 (m, 4H, C²H₂/N-CH₂) 1.53 (m, 2H, CH₂) 1.33 (m, 2H, CH₂) 0.91 (t, 3H, CH₃) |
| L-NPh⁶ | 6.38 (s, 1H, NH) | 4.20 (t, 2H, C²H₂) 3.76 (t, 2H, C²H₂) 7.60 (m, 2H, o-H) 7.42 (m, 2H, m-H) 7.27 (m, 2H, p-H) |
| purSH₂ | 13.70 (s; 2H, N⁵⁹H) | 8.33 (s, 1H, C²H) 8.14 (s, 1H, C²H) |
| tucH₂ | 12.37 (s; 2H, N⁴¹H) | 7.36 (d; 1H, C²H) 5.78 (d; 1H, C²H) |
| pymSH² | 8.23 (s; 1H, N⁵H) | 8.65 (d; 1H, C²H) 7.32 (m; 1H, C²H) 6.79 (t; 1H, C²H) |
| pySH² | 13.46 (s; 1H, N⁵H) | 7.65 (s, 1H, C²H) 7.35 (t; 1H, C²H) 7.25 (t; 1H, C²H) |
| PPh₃ | — | 7.32 (m; o-H, m-H, p-H) 15H |
| dpmm | — | 2.18 (s; 2H, P-CH₂-P) 7.44 (m, 8H, o-H) 7.30 (m, 12H, m-H, p-H) |

*a* See ESI for spectra. ³ (N-CH₂) and (ring proton C²H₂ or C²H₃) got merged. ⁴ CDCl₃. ⁵ DMSO. ⁶ N-Ph protons obscured by phenyl ring of PPh₃.
shown low field shift relative to the free co-ligand. All these variations showed that it is the ipso carbon that is most affected in all the coordination compounds and hence the coordination of the PPh₃ to the silver(i) is through P donor atom. This upfield shift is attributed to electromeric effect involving movement of p-C and m-C phenyl ring electrons towards i-C-P bond axis leading to shielding of this carbon. The ¹³C (¹³C-³¹P) coupling constant did not show regular trend.

### Molecular structures

The crystal data of complexes 2, 3, 5–8 are given Table 4 which is placed in the experimental section, and their important bond parameters are given in Table 5. Complexes 3, 5 and 6 crystallized in monoclinic crystal system in space groups P2₁/c [3], C2/c (5) and P2₁/n (6), while each of complexes 2, 7 and 8 crystallized in triclinic crystal system in space group PI.

The molecular structures of complexes, [Ag(S-L-NPr⁺)[PPh₃]₂(O–NO₂)] 2, and [Ag(S-L-NBu⁺)[PPh₃]₂(O–NO₂)] 3 are given in Fig. 1 and 2 respectively. In complex 2, silver(i) is bonded to one S donor atom of the L-NPr⁺ thio-ligand, two P donor atoms of PPh₃ ligands and one O donor atom of a nitrate ion at bond distances of 2.616(13) Å, 2.4552(11), 2.4450(11) Å and 2.496(4) Å respectively (Table 5). The corresponding bond distances of complex 3 are 2.5340(7) Å, 2.4524(6), 2.455(6) Å and 2.4999(19) Å, respectively. These bond distances are less than the sum of the covalent radii of the silver atom and the donor group (Ag–S, 3.50, Ag–P, 3.55, Ag–O, 3.20 Å) and thus bonds in these complexes are similar to those found in literature. While Ag–P and Ag–O distances in two complexes are similar, the corresponding Ag–S distances are different. The n-butyln group at N atom of thio-ligand L-NBu⁺ with positive inductive effect appears to increase lewis basicity of this ligand, which makes relatively short Ag–S bond in complex 3 versus that in 2 where it is longer. The short Ag–S distance in 3 is followed by relatively longer C–S bond distance (1.695(6) Å, 2, 1.708(3) Å, 3). The differences in Ag–S bond distances in these two complexes are reflected in Ag–O distances which is marginally short in 2. The O–N bond distances of ONO₂ group differ more and lie in the range, 1.175 to 1.284 Å, while those in complex 3, these distances are 1.232(3) to 1.249(3) Å – short in complex 2 (1.175(7) Å) versus that in 3 (1.232(3) Å). The bond angles around the Ag metal center are in the ranges 97–129° (2) and 101–123° (3) respectively, suggesting distorted tetrahedral geometry of these complexes.

### Table 3  The ¹³C NMR spectral data (δ in ppm) of complexes 1, 3, 5–8 and free ligands

| Complex/free ligand | Carbons of thio-ligands | Other ring/chain carbons | Carbons of co-ligands (PPh₃/dppm) |
|---------------------|-------------------------|--------------------------|----------------------------------|
| [Ag(S-L-NEt)[PPh₃]₂(O–NO₂)] 1⁺ | 179.9 | 48.26 (C⁶), 42.06 (N–CH₂), 41.06(C⁶), 133.91 (i-C, J_C–P = 60 Hz), 132.47 (o-C, J_C–P = 135 Hz), 129.98 (p-C), 128.70 (m-C, J_C–P = 40 Hz) | i-C, o-C, m-C, p-C |
| [Ag(S-L-NBu⁺)[PPh₃]₂(O–NO₂)] 3⁺ | 180.4 | 48.80 (C⁶), 46.72 (N–CH₂), 41.89 (C⁵), 29.24 (CH₂), 19.88 (CH₃), 13.78 (CH₃) | |
| [Ag₂(N,S-purSH₂)₂[μ-dppm]₂(NO₃)] 5⁺ | 171.6 | 145.91(C⁵), 133.34(C⁴/C₈), 128.75(C⁵) | |
| [Ag₂(N,S-purSH₂)₂[μ-dppm]₂(NO₃)] 6⁺ | 183.6 | 152.75(C⁵), 133.63(C⁴/C₈), 129.25(C⁵) | |
| [Ag(S-tucH₂)[PPh₃]₂(NO₂)] 7⁺ | 163.6 | 164.92(C⁹), 133.64(C⁶), 100.76(C⁶) | |
| [Ag(N,S-pymS)[PPh₃]₂] 8⁺ | 183.2 | 156.16(C⁴H/C₇H), 114.16(C⁵H) | |
| L-Net⁺ | 182.9 | 47.97(C⁶), 41.67 (N–CH₂), 41.39(C⁶), 12.21(CH₃) | |
| L-NBu⁺,b | 183.4 | 15.47(C⁹), 46.77 (N–CH₂), 41.44(C⁹), 29.26(CH₃), 19.99 | |
| (CH₃), 13.89(CH₃) | dpmp⁺: 138.88 (i-C, J_C–P = 40 Hz), 132.92 (o-C, J_C–P = 84 Hz), 128.80 (p-C), 128.48 (m-C, J_C–P = 28 Hz), 28.12 (CH₃) | |
| purSH₂⁺ | 171.4 | 152.08(C⁷), 145.04(C⁴/C₈), 128.76(C⁵) | |
| tucH₂⁺ | 161.5 | 176.17(C⁴), 142.63(C⁶), 106.80(C₃) | |
| pymSH⁺ | 181.9 | 154.69(C⁵), 159.06(C₄), 110.01(C₇H) | |

See ESI for spectra. ¹⁷CDCl₃, ¹⁹DMSO.
Table 4  Crystallographic data for complexes 2, 3, 5–8

|   | 2                  | 3                  | 5                  |
|---|--------------------|--------------------|--------------------|
|   | Empirical formula  | $C_{42}H_{42}AgN_3O_3P_2S$ | $C_{42}H_{42}AgN_3O_3P_2S$ | $C_{43}H_{42}AgN_3O_3P_2S$ |
| CCDC | 1878467           | 1878471            | 1878472            |
| $M$  | 838.65            | 852.68             | 1448.88            |
| $T/K$ | 293(2)            | 173(2)             | 293(2)             |
| $\lambda$ Å | Cu-Kz, 1.54178 | Mo-Kz, 0.71073     | Cu-Kz, 1.54178     |
| Crystal system | Triclinic          | Monoclinic         | Monoclinic         |
| Space group | $P1$              | $P2_1/c$           | $C2/c$             |
| $a$ (Å) | 10.5641(6)        | 13.6628(4)         | 25.2895(7)         |
| $b$ (Å) | 12.9718(7)        | 16.2436(5)         | 13.2208(2)         |
| $c$ (Å) | 15.3593(8)        | 18.8410(6)         | 23.5888(7)         |
| $\alpha$ (°) | 77.698(4)        | 90                 | 90                 |
| $\beta$ (°) | 82.402(4)        | 105.902(3)         | 125.671(4)         |
| $\gamma$ (°) | 79.796(4)        | 90                 | 90                 |
| $V$ (Å³) | 2014.12(19)      | 4021.4(2)          | 6407.1(4)          |
| $Z$  | 2                 | 4                  | 4                  |
| $D_{\text{calcd}}$ (g cm⁻³) | 1.383             | 1.408              | 1.502              |
| $\mu$ (mm⁻¹) | 5.577            | 0.675              | 6.958              |
| $F(000)$ | 864               | 1760               | 2944               |
| Refns collected | 14 231           | 36 999             | 12 856             |
| Unique refinns | 7658 ($R_{\text{int}} = 0.0443$) | 13 487 ($R_{\text{int}} = 0.0418$) | 6067 ($R_{\text{int}} = 0.0264$) |
| Data/restraints/parameters | 7658/0/480 | 13 487/0/479 | 6067/223/447 |
| Refines with $| I > 2\sigma(| I |$] | 6347             | 9990              | 5309               |
| Final $R$ indices $| I > 2\sigma(| I |$] | $R_1 = 0.0548$ | $R_1 = 0.0449$ | $R_1 = 0.0383$ |
|           | $wR_2 = 0.1389$ | $wR_2 = 0.0962$ | $wR_2 = 0.1040$ |
| Final $R$ indices (all data) | $R_1 = 0.0668$ | $R_1 = 0.0702$ | $R_1 = 0.0467$ |
|           | $wR_2 = 0.1469$ | $wR_2 = 0.1121$ | $wR_2 = 0.1101$ |
| Largest diff. peak/hole e Å⁻³ | 1.720 and −0.671 | 1.193 and −0.482 | 1.160 and −0.441 |

|   | 6                  | 7                  | 8                  |
|---|--------------------|--------------------|--------------------|
|   | Empirical formula  | $C_{40}H_{42}AgN_3O_3P_2S$ | $C_{40}H_{42}AgN_3O_3P_2S$ | $C_{43}H_{42}AgN_3O_P_5S$ |
| CCDC | 1878468           | 1878469            | 1878470            |
| $M$  | 846.60            | 840.59             | 775.59             |
| $T/K$ | 293(2)            | 293(2)             | 173(2)             |
| $\lambda$ Å | Cu-Kz, 1.54178 | Cu-Kz, 1.54178     | Mo-Kz, 0.71073     |
| Crystal system | Monoclinic          | Triclinic         | Triclinic         |
| Space group | $P2_1/n$          | $P1$              | $P1$              |
| $a$ (Å) | 9.2210(3)         | 12.5062(7)         | 10.1240(4)         |
| $b$ (Å) | 25.4962(8)        | 13.1744(7)         | 13.4635(6)         |
| $c$ (Å) | 17.0609(5)        | 15.1280(7)         | 14.1829(6)         |
| $\alpha$ (°) | 90                | 99.534(4)          | 77.466(4)          |
| $\beta$ (°) | 100.830(3)       | 103.826(5)         | 78.784(4)          |
| $\gamma$ (°) | 90                | 114.897(5)         | 78.993(4)          |
| $V$ (Å³) | 3939.6(2)         | 2092.7(2)          | 1828.62(14)        |
| $Z$  | 4                 | 2                  | 2                  |
| $D_{\text{calcd}}$ (g cm⁻³) | 1.427             | 1.334              | 1.409              |
| $\mu$ (mm⁻¹) | 5.728            | 5.408              | 0.730              |
| $F(000)$ | 1728             | 860                | 796                |
| Refns collected | 16 236            | 15 670             | 21 420             |
| Unique refinns | 7485 ($R_{\text{int}} = 0.0407$) | 7954 ($R_{\text{int}} = 0.0586$) | 12 042 ($R_{\text{int}} = 0.0323$) |
| Data/restraints/parameters | 7485/0/482 | 7954/0/472 | 12 042/0/436 |
| Refines with $| I > 2\sigma(| I |$] | 6025             | 6920              | 9569               |
| Final $R$ indices $| I > 2\sigma(| I |$] | $R_1 = 0.0464$ | $R_1 = 0.0449$ | $R_1 = 0.0393$ |
|           | $wR_2 = 0.1153$ | $wR_2 = 0.1161$ | $wR_2 = 0.1125$ |
| Final $R$ indices (all data) | $R_1 = 0.0519$ | $R_1 = 0.0576$ | $R_1 = 0.0575$ |
|           | $wR_2 = 0.1226$ | $wR_2 = 0.1339$ | $wR_2 = 0.1339$ |
| Largest diff. peak/hole e Å⁻³ | 1.114 and −0.612 | 0.745 and −0.972 | 0.808 and −0.488 |

is found that O1–Ag–S angle is smallest, 97.59(13), while P–Ag–P angle is largest, 129.48(4). It is opposite in complex 3 and these differences are attributed to the steric effect of R groups at N atoms of L-NR moiety.

Purine-6-thione chelates to Ag(I) in complex [Ag(N,S-purSH)₂][PPh₃]₂][NO₃]₆ (Fig. 3), through its N₇, S-donor atoms with Ag–N₇ and Ag–S bond distances of 2.468(4) and 2.6478(10) Å respectively. The Ag–P bond distances of 2.4511(8), 2.4301(9) Å.
˚A are similar to those found in complex 2. The nitrate NO$_3^-$ ion is not coordinated in complex 6 with O–N bond distances of 1.200(6), 1.218(6) and 1.240(5) ˚A. It is noted that the average O–N distance of 1.219(6) ˚A in complex 6 is less than the similar average O–N distances of 1.229(7) ˚A in complex 2 and 1.238(3) ˚A in complex 3. The difference is obviously due to the coordinated nitrate in 2 and 3, and ionic in complex 6 (Fig. 3). The N$^7$–Ag–S bite angle of 79.87(9)$^\circ$ is smallest, while P–Ag–P angle of 130.17(3)$^\circ$ is the largest among angles around the Ag atom. The geometry is severely distorted from the tetrahedron.

When dppm was used in place of PPh$_3$, purine-6-thione with silver(i) nitrate has yielded a P,P-bridged dinuclear complex,
angles around Ag vary from 77.27(7) to 143.54(2)° lying outside the coordination sphere of silver metal ion. The thio-ligand purSH₂ is chelating through its N₁,S₁ donor atom at Ag–S distance of 2.5464(9) Å and two P-donors at Ag–P bond distances of 2.4531(7) and 2.4787(8) Å (Fig. 5). The angles around the central silver atom in the range of 110–124° suggest distorted trigonal planar arrangement. The nitrate is lying outside the coordination sphere. The pyrimidine-2-thione ligand chelates to the silver atom as an anionic ligand through its N¹,S¹ donor atoms in [Ag(N,S-pymS)(PPh₃)₂]NO₃ 8 (Fig. 6). Here, the Ag–S bond distance {2.5502(6) Å} is smaller than that found in the analogous complex [Ag(S-pymSH)(PPh₃)₂]NO₃, owing to pyrimidine-2-thione as anionic ligand in former complex versus neutral ligand in the latter complex. Other bond distances, namely, Ag–P and Ag–N bond distances are in same trend as found in complex 6 with Ag–N bonds. The angles around Ag vary in the range, 61–125° with N–Ag–S bite angle of 61.89(5)° and P–Ag–P bond angle of 125.17(2)° suggesting distorted tetrahedral geometry of complex 8. The C–S bond lengths in complexes 2, 3, 5–8 fall in the range 1.68–1.72 Å, and are less than the C–S single bond length (1.81 Å) but longer than the C–S double bond length (1.62 Å), suggesting a partial double bond character in the C–S bonds in complexes.²³

ESI-mass studies. The ESI-mass spectra of some selected complexes (1–5) was recorded and four types of species were identified (shown in Chart 3). Where type A species is, [Ag(L)(PR)]²⁺ {1–5} {L = L-NEt (1), L-NPr (2), L-NBu (3), L-NPPh(4); PR = PPh₃ (1–4), dppm (5)}, type B species is, [Ag(L-NR)₂]²⁻ {1–4} {R = Et (1), Pr (2), Bu (3), Ph(4)}, type C species is, [Ag(PR)₃]⁻ {1–5} {PR = PPh₃ (1–4), dppm (5)} and type D species is, [Ag(PR)]⁰ (5) (PR = dppm). Molecular ion species were not shown by these complexes and all of the complexes have shown loss of a nitrate ion (see ESI, Fig. S60–S73).  

Antimicrobial studies

Silver complexes with imidazolidine-2-thiones and benzimidazoline-2-thione. Silver complexes of imidazolidine-2-thiones and benzimidazoline-2-thione, namely, 1–4, 10–12 have shown bio-activity against Gram positive bacteria meticillin resistant *Staphylococcus aureus* (MRSA) with zone of inhibition values (zoi) in the range 14–30 mm. Complexes 1 and 4 have lower activity (22–23 mm zoi) but their MIC (10 μg mL⁻¹) value is same as that of gentamicin. In contrast, complex 10 has same activity as that of the standard drug gentamicin (33 mm zoi), with low MIC value (5 μg mL⁻¹) (Tables 6 and 7). The activity of analogous silver halide complexes of imidazolidine-2-thiones was found to be in a lower range, 15–25 mm zoi.⁴⁴ Further, all of these complexes, except complex 12, were found to be active against Gram positive bacteria *Staphylococcus aureus* (MTCC 740) with zoi values in the range 12–24 mm. The activity of complexes 1, 10 and 11 was comparable (22–24 mm zoi) to that of gentamicin (26 mm zoi) but their MIC values were somewhat high (Table 7). The activity of silver nitrate complexes is comparable to that of analogous silver halide complexes.⁴⁴
Further, it is interesting to note that complexes 1, 2 and 11 have shown activity of 24 mm (2) and 30 mm (1, 11) against Gram positive bacteria *Staphylococcus epidermidis* (MTCC 435), with low MIC values (5–10 μg mL\(^{-1}\)) in comparison to the standard drug gentamicin with low activity and high MIC (zoi = 25 mm; MIC, 30 μg mL\(^{-1}\)).

Complexes 1–3, 10 and 11 have shown activity against the microorganism Gram positive bacteria *Enterococcus faecalis* (MTCC 439) in the range 22–34 mm. Significantly, three complexes (2, 3 and 11) have more activity (zoi, 30–34 mm) with low MIC values (1–7 μg mL\(^{-1}\)) than that of gentamicin (zoi, 27 mm, 30 μg mL\(^{-1}\)). The antimicrobial activity against Gram negative bacteria *Shigella flexneri* (MTCC 1457) in the range 19–24 mm (zoi) has been shown by the complexes 1, 3, 4 and 11 with MIC values in the range 7–50 μg mL\(^{-1}\). This activity is lower than that shown by the standard drug (zoi, 34.5 mm, MIC, 5 μg mL\(^{-1}\)). Complexes 1, 2, 10 and 11 are active against yeast.
Candida albicans (MTCC 227) (21–26 mm) with MIC values in the range 5–50 μg mL⁻¹. This activity is lower than that of the standard drug amphotericin B (zoī, 34 mm; MIC, 0.1 μg mL⁻¹).

Only one complex showed activity of 26 mm with MIC value of 5 μg mL⁻¹, which is relatively close to that of the reference compound. Finally, it is added here that these complexes are more active as compared with the uncoordinated thio-ligands.

Silver complexes with purine-6-thione and related ligands.
Silver complexes 5–9 have shown activity against Gram positive bacteria methicillin resistant Staphylococcus aureus (MRSA) in

Table 6 Antimicrobial activity of complexes 1–12a,b,c

| Complex/free thio-ligand/standard drug | MRSAa | S. aureusb | S. epidermidisc | E. faecalisbd | S. flexnerie | C. albicansf |
|---------------------------------------|-------|------------|----------------|---------------|--------------|-------------|
| Imidazolidine-2-thione (1–4, 10, 11) and benzimidazole-2-thione (11) complexes |       |            |                |               |              |             |
| [Ag(S-L-NEt)[PPh₃]₂(O-NO₂)] 1        | 22    | 23         | 30             | 23            | 19           | 22          |
| [Ag(S-L-NPr)₉(PPh₃)₂(O-NO₂)] 2       | 24    | 20         | 24             | 30            | 13           | 21          |
| [Ag(S-L-NBu)₉(PPh₃)₂(O-NO₂)] 3       | 14    | 13         | 15             | 30            | 23           | 14          |
| [Ag(S-L-NPh)₉(PPh₃)₂(O-NO₂)] 4       | 22    | 19         | 20             | 19            | 24           | 18          |
| [(Ag₂(N,N-purSH₂)(PPh₃)₂(NO₃)] 5     | 12    | 17         | 12             | 15            | 12           | 13          |
| (Ag₂(N,N-purSH₂)(PPh₃)₂(NO₃)] 6     | 20    | 19         | 14             | 13            | 12           | 12          |
| [Ag(S-tucH₂)[PPh₃]NO₃] 7             | 12    | 12         | 17             | NA            | NA           | 13          |
| [Ag(S-pymS)[PPh₃]NO₃] 8              | 19    | 19         | 13             | NA            | NA           | 13          |
| purSH₂                               | 18    | 13         | NA             | NA            | NA           | 13          |
| tucH₂                                | 19    | 16         | NA             | NA            | NA           | 12          |
| PySH                                 | 19    | 17         | 17             | NA            | 20           | 23          |
| dpmm                                 | 19    | 19         | 13             | NA            | 14           | 30          |
| Gentamicin [d,k]                      | 34d   | 26d        | 25d            | 27d           | 34.5d        | —           |
| Amphotericin B [d,k]                  | —     | —          | —              | —             | —            | —           |

a All measurements are in mm diameter of the inhibition zone (N.A. indicates no activity). b The standard deviation varied in the range 0–1 based on three readings. c Studies were made in DMSO. d Commercially available antimicrobial agents. e MRSA. f Staphylococcus aureus. g Staphylococcus epidermidis. h Enterococcus faecalis. i Shigella flexneri. j C. albicans. k Gentamicin acts as positive control against bacteria (MRSA, S. aureus, S. epidermidis, E. faecalis, S. flexneri) and amphotericin B acts as positive control against yeast, C. albicans.
Table 7 Minimum inhibitory concentration (µg mL⁻¹) of silver(II) complexes 1–12<sup>a,b</sup>

| Complex/standard drug | MRSA | S. Aureus | S. epidermidis | E. faecalis | S. flexneri | C. albicans |
|-----------------------|------|-----------|--------------|------------|------------|------------|
| Imidazolidine-2-thione (1–4, 10, 11) and benimidazoline-2-thione (11) complexes | | | | | | |
| 1 | 10 | 10 | 5 | 10 | 50 | 50 |
| 2 | 50 | 50 | 10 | 7 | ND | 1010 |
| 3 | ND | ND | ND | 1 | 7 | ND |
| 4 | 10 | 500 | 50 | 500 | 10 | 750 |
| 5 | 5 | 10 | 50 | 50 | 1000 | 5 |
| 10 | 7 | 10 | 7 | 1 | 50 | 10 |
| 11 | 7 | ND | ND | ND | ND | ND |
| 12 | 7 | ND | ND | ND | ND | ND |

| Purine-6-thione, 2-thiouracil, pyrimidine-2-thione and pyridin-2-thione complexes (6–9) | | | | | | |
| 5 | ND | ND | ND | ND | ND | 50 |
| 6 | 1250 | ND | ND | ND | ND | 50 |
| 7 | 50 | ND | ND | ND | 7 | 10 |
| 8 | ND | ND | ND | ND | ND | ND |
| 9 | 50 | ND | ND | ND | ND | 50 |
| Gentamicin | 10 | 0.5 | 30 | 30 | 5 | — |
| Ampotericin B | — | — | — | — | — | 0.1 |

<sup>a</sup> MIC in µg mL⁻¹. <sup>b</sup> ND—not determined.

The OD values of silver complexes (1, 3, 4, 7, 8, 11) at wavelength 590 nm are as follows:

| Complexes | OD values | Cell viability |
|-----------|-----------|---------------|
| [Ag(L-NEt)(PPh₃)₂O(NO₂)]<sub>2</sub> | 0.237<sup>a</sup> | 79.53% |
| [Ag(L-NBu<sub>3</sub>)(PPh₃)₂O(NO₂)]<sub>2</sub> | 0.224<sup>a</sup> | 75.17% |
| [Ag(L-NPh)(PPh₃)₂O(NO₂)]<sub>2</sub> | 0.288<sup>a</sup> | 96.31% |
| [Ag(tucH₂)(PPh₃)₂NO₃] | 0.565<sup>a</sup> | 84% |
| [Ag(pymS)(PPh₃)₂] | 0.666<sup>a</sup> | 99.10% |
| [Ag(L-NMe₂)(PPh₃)₂NO₃] | 0.271<sup>a</sup> | 90.94% |

<sup>a</sup> OD value of control = 0.298. <sup>b</sup> OD value of control = 0.672.

Similarly, pySH was more active (zoi = 18 mm) than its complex 9 (zoi = 14 mm).

Only complex 9 showed activity (zoi = 13 mm) against Gram positive bacteria Enterococcus faecalis (MTCC 439), while other complexes and free ligands were inactive. Among the free thioligands, only pymSH and pySH showed activity of 20 and 14 mm (zoi) against Gram negative bacteria Shigella flexneri (MTCC 1457), but as regards their complexes, only 2-thiouracil complex 7 showed activity of 26 mm (zoi) which was somewhat close to that of standard drug gentamicin (zoi = 34.5 mm) (Table 7). Additionally, their MIC values were comparable (complex 7, MIC = 7 µg mL⁻¹; gentamicin, MIC = 7 µg mL⁻¹). This is an interesting outcome of the study owing to the importance of tucH₂ derivatives in the development of metal based drugs.42–44

Finally, as regards activity against the yeast Candida albicans, the thi-ligand purSH₃ was inactive, while its complexes 5 and 6 showed moderate activity of 17 mm (5) and 18 mm (6) respectively. Further, while free tucH₂ was inactive, its complex 7 was found to be active with (zoi = 20 mm). In contrast, the thioligands pymSH (23 mm) and pySH (30 mm) were found to be more active than their corresponding complexes 8 (13 mm) and
formazan product which is measured spectrophotometrically. Interestingly, complexes $[\text{Ag}(\text{L-NEt})(\text{PPh}_3)_2(\text{O-NO}_2)]$ 1, $[\text{Ag}(\text{L-NBu}')(\text{PPh}_3)_2(\text{O-NO}_2)]$ 3, $[\text{Ag}(\text{L-NPH})(\text{PPh}_3)_2(\text{O-NO}_2)]$ 4, $[\text{Ag}(\text{L-tucH}_2)(\text{PPh}_3)_2(\text{NO}_2)]$ 7, $[\text{Ag}(\text{N,S-pymS})(\text{PPh}_3)_2]$ 8 and $[\text{Ag}(\text{L-NMe})_2(\text{PPh}_3)]$ showed purple color with optical density (OD) of 0.237 (1), 0.224 (3), 0.288 (4), 0.271 (11) (control OD = 0.298), 0.565 (7) and 0.666 (8) (control = 0.672) respectively at 590 nm wavelength. The OD of the control (untreated cells in DMSO) was 0.872 (Table 8). Therefore these complexes were found to be non-cytotoxic with respective cell viability of 79.53% (complex 1), 75.17% (complex 3), 96.31% (complex 4), 84% (complex 7), 99.10% (complex 8) and 90.94% (complex 11).

**Antitumor activity**

A status of anti-cancer/tumor activity of complexes of silver(l) with heterocyclic-2-thiones is given in introduction,17,18,36,39 and it was noted that the study pertained to *in vitro* cytotoxic activity against murine leukemia (L1210), human T-lymphocyte (Molt4/C8 and CEM) cells, breast cancer cell lines (MDA-MB-231, MCF-7), colon cancer cell line (HT-29), and leiomysarcoma cancer cells (LMS). In this investigation, silver(l) complexes (1–7, 9–10) with the thio-ligands, namely, N-substituted-imidazolidine-2-thiones, benzimidazole-2-thione, purine-6-thione, pyridine-2-thione and 2-thiouracil have been studied for their *in vitro* antitumor activity against human bone cancer cell line MG63, using MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay. The antitumor potential of these complexes was evaluated by exposing a fixed number of cancer cells to increasing concentration of complexes. Fig. 7 and 8 depict the effect of complex concentration on the death of the cancerous cells. As an example, complex 2 at concentrations of 6.25, 12.5, 25, 50 and 100 μM, showed the percent killing of cancerous cells up to 40, 65, 75, 85 and 91% respectively. Similar effect was observed when complexes 1, 3–7, 9–10 were studied against MG63 cancerous cells. Further, the cytotoxic activity of complexes on cancerous cell line is interpreted on the basis of their IC$_{50}$ (half maximal inhibitory concentration) values (Table 8). The IC$_{50}$ values of different complexes and the free thio-ligands were calculated from the curves obtained between concentration of complexes and percentage viability of cancerous cells (Fig. 9).

The IC$_{50}$ values of complexes 1–12 ranged between 6.42–29.22 μM (Table 9). Among the complexes tested, complexes, 2, 3, 5 and 10 have shown IC$_{50}$ values of 6.42 (2), 9.21 (3), 7.62 (5) and 8.17 (10) μM respectively; other complexes 1, 6, 7, 9 and 11

![Fig. 8](image8.png)  
**Fig. 8** Effect of concentration of complexes 6, 7, 9–11 on viability of MG 63 cancerous cells; where untreated cells represent control.

![Fig. 9](image9.png)  
**Fig. 9** Plot of IC$_{50}$ values of complexes 1–6, 7, 9–11 against MG 63 cells.



Table 9  Anticancer activity of silver complexes (1–7, 9–11) against human bone cancer MG63 cell line$^a$

| Complexes | IC$_{50}$ (μM) | IC$_{50}$ (μM) |
|-----------|---------------|---------------|
| $[\text{Ag}(\text{L-NH})(\text{PPh}_3)_2](\text{O-NO}_2)]$ 1 | 13.20 | 14.58 |
| $[\text{Ag}(\text{S-pySH})(\text{PPh}_3)_2]$ 6 | 13.33 |
| $[\text{Ag}(\text{S-N,S-pymS})(\text{PPh}_3)_2]$ 7 | 15.80 |
| $[\text{Ag}(\text{N,S-pyrH})(\text{PPh}_3)_2]$ 8 | 8.17 |
| $[\text{Ag}(\text{L-NMe})_2(\text{PPh}_3)](\text{NO}_2)$ 10 | 6.42 |
| $[\text{Ag}(\text{N,S-purSH})(\text{PPh}_3)_2](\text{O-NO}_2)]$ 5 | 7.62 |

$^a$ Ligands: L-NR, R = H, Me, Et, Pr$^a$. Bu$^a$ and Ph; PPh$_3$, IC$_{50}$ (μM) is >100; purSH$_2$, 24.78, tucH$_2$, 44.26 and pySH, 29.66.
have IC_{50} values in close range, 13.22 to 16.61 μM, and finally complex 4 lowest IC_{50} value of 29.22 μM. The free thio-ligands, L-NR (R = H, Me, Et, Pr, Bu, and Ph), purSH_2, tucH, and pymSH are either inactive or have low ant-cancer activity.

**Conclusion**

Coordination compounds of N-substituted 1,3-imidazolidine-2-thiones, purge-6-thione, 2-thiouracil, pyrimidine-2-thione and pyridine-2-thione with silver(I) nitrate have shown moderate to high antimicrobial bio-activity against methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Staphylococcus epidermidis*, and yeast *Candida albicans*. Coordination compounds [Ag(S-L-NEt)(PPh_3)_2][NO_3] 1, [Ag(S-L-NPh)(PPh_3)_2][NO_3] 4, [Ag(S-tucH)(PPh_3)_2][NO_3] 7, [Ag(N,S-pymS)(PPh_3)_2][NO_3] 8 and [Ag(S-L-NMe)(PPh_3)][NO_3] 11 studied for their in vitro cell viability were found to be non-cytotoxic with respective cell viability of 79.53 (1), 96.31 (4), 84 (7), 99.10 (8) and 90.94% (11) respectively. Specifically complexes of N-substituted imidazolidine-2-thiones have shown unusual bio-activity against methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and *Enterococcus faecalis* which rivals with gentamicin or even better in some cases. The anti-tumor study of silver complexes against human which rivals with gentamicin or even better in some range 6 and co-ligands develop chemical environments which lead to enhanced lipophilic character of complexes, in comparison to silver nitrate which being soluble in water combines with chloride in body system forming insoluble AgCl, thus losing its bioactivity. However, presence of nitrate in ionic/coordinated forms in the present mixed ligand complexes of thio-ligands either inactive or have low ant-cancer activity.

**Experimental section**

**Chemicals and techniques**

The thio-ligands, namely, purge-6-thione (purSH_2), pyrimidine-2-thione (pymSH), pyridine-2-thione (pySH), 2-thiouracil (tucH) and benz-imidazoline-2-thione (bzmSH_2) were procured from Sigma-Aldrich Ltd and used as such, whereas, other thio-ligands such as, imidazoline-2-thione (L-NH), 1-methyl-imidazoline-2-thione (L-NMe), 1-ethyl-imidazolidine-2-thione (L-NEt), 1-n-propyl-imidazoline-2-thione (L-NPr^+), 1-n-butyl-imidazoline-2-thione (L-NBu^+), 1-phenyl-imidazoline-2-thione (L-NPh) were prepared as per the literature methods. Silver(I) nitrate was purchased from Sigma-Aldrich Ltd and used as such, whereas, 1-n-butyl-1,3-imidazolidine-2-thione (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid 1-n-ethyl-imidazoline-2-thione (0.038 g, 0.30 mmol) and the contents were stirred for a period of 1 h at room temperature. To the clear solution formed was added triphenylphosphine (0.076 g, 0.30 mmol) and slow evaporation of this solution at room temperature for a period of one week gave white crystal-solid of complex 1 (yield, 0.096 g, 80%, mp 87–90 °C). Elemental analysis for AgC_{43}H_{44}N_{8}P_{4}S_{2}O_{8} (824): calcd C 59.72; H 4.85; N 5.10; S 3.88%; found: C 59.97; H 5.13; N 5.17, S 4.16%. Complex 4 was prepared similarly.

[Ag(L-NPh)_{2}(PPh_3)(ONO_3)] (3). To a solution of silver(I) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid 1-n-propyl-imidazoline-2-thione (0.042 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added triphenylphosphine (0.076 g, 0.30 mmol). Slow evaporation of this solution at room temperature formed a white solid which after re-crystallization from dichloromethane and toluene mixture (5 mL, 4:1, v/v) yielded colorless crystals of 2 (yield, 0.086 g, 70%, mp 153–156 °C). Elemental analysis for AgC_{42}H_{42}N_{8}P_{4}S_{2}O_{8} (838.65): calcd C 60.09; H 5.00; N 5.00; S 3.81%; found: C 60.35; H 5.25; N 5.37, S 4.12.

[Ag(L-NBu)(PPh_3)(ONO_3)] (4). Colorless compound (yield, 0.099 g, 78%, mp 136–138 °C). Elemental analysis for AgC_{45}H_{40}N_{8}P_{4}S_{2}O_{8} (873.24): calcd C 56.51; H 5.16; N 4.92; S 3.75%; found: C 56.77; H 5.56; N 5.06; S 3.67%.

**Synthesis of Silver(i) complexes**

[Ag(L-NEt)(PPh_3)_2(ONO_3)] (1). To a solution of silver(I) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid 1-n-ethyl-imidazoline-2-thione (0.038 g, 0.30 mmol) and the contents were stirred for a period of 1 h at room temperature. To the clear solution formed was added triphenylphosphine (0.076 g, 0.30 mmol) and slow evaporation of this solution at room temperature for a period of one week gave white crystal-solid of complex 1 (yield, 0.096 g, 80%, mp 87–90 °C). Elemental analysis for AgC_{43}H_{44}N_{8}P_{4}S_{2}O_{8} (824): calcd C 59.72; H 4.85; N 5.10; S 3.88%; found: C 59.97; H 5.13; N 5.17, S 4.16%. Complex 4 was prepared similarly.

[Ag(L-NPh)(PPh_3)(ONO_3)] (2). To a solution of silver(I) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid 1-n-propyl-imidazoline-2-thione (0.042 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added triphenylphosphine (0.076 g, 0.30 mmol). Slow evaporation of this solution at room temperature formed a white solid which after re-crystallization from dichloromethane and toluene mixture (5 mL, 4:1, v/v) yielded colorless crystals of 2 (yield, 0.086 g, 70%, mp 153–156 °C). Elemental analysis for AgC_{42}H_{42}N_{8}P_{4}S_{2}O_{8} (838.65): calcd C 60.09; H 5.00; N 5.00; S 3.81%; found: C 60.35; H 5.25; N 5.37, S 4.12.

[Ag(L-NBu)(PPh_3)(ONO_3)] (3). To a solution of silver(I) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid 1-n-butyl-1,3-imidazolidine-2-thione (0.046 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added triphenylphosphine (0.076 g, 0.30 mmol). Slow evaporation of this solution at room temperature formed a white solid which after re-crystallization from dichloromethane and toluene mixture (5 mL, 4:1, v/v) yielded colorless crystals of 3 (yield, 0.093 g, 75%, mp 118–120 °C). Elemental analysis for AgC_{43}H_{44}N_{8}P_{4}S_{2}O_{8} (852.68): calcd C 60.56; H 5.16; N 4.92; S 3.75%; found: C 60.77; H 5.56; N 5.06; S 3.67%.

**[Ag(L-NPh)(PPh_3)(ONO_3)] (4).** Colorless compound (yield, 0.099 g, 78%, mp 136–138 °C). Elemental analysis for AgC_{45}H_{40}N_{8}P_{4}S_{2}O_{8} (873.24): calcd C 61.94; H 4.59; N 4.82; S 3.67%; found: C 49.59; H 4.26; N 9.51, S 4.08%.
[Ag(purSH)₂(PPh₃)₂][NO₃] (6). To a solution of silver(i) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid triphenylphosphine (0.076 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added a solution of pyrimidine-2-thione (0.016 g, 0.15 mmol) in methanol (5 mL) in presence Et₃N base (0.5 mL). Stirred for 1 h at room temperature. To the clear solution formed was added solid triphenylphosphine (0.076 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added solid 2-thiouracil (0.037 g, 0.30 mmol) and slow evaporation of this solution at room temperature gave colorless crystals of 6 (yield, 0.077 g, 65%, mp 170 °C). Elemental analysis for Ag₄H₃N₄P₂S₂O₃ (846.60): calcd C 57.73; H 4.15; N 3.73%; found: C 57.73; H 4.15; N 3.73%.

[Ag(tucH₂)(PPh₃)₂][NO₃]·H₂O (7). To a solution of silver(i) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid triphenylphosphine (0.076 g, 0.30 mmol) and slow evaporation of this solution at room temperature gave pale yellow crystals of 7 (yield 0.12 g, 92%, mp 116–118 °C). Elemental analysis for Ag₉H₃₆N₄P₂S₂O₆ (1180.92): calcd C 63.08; H 5.15; N 4.93, S 3.76%. Complex 9 was prepared similarly.

[Ag(pymS)(PPh₃)₂][CH₃OH] (8). To a solution of silver(i) nitrate (0.025 g, 0.15 mmol) in methanol (5 mL) was added solid triphenylphosphine (0.076 g, 0.30 mmol) and the contents were stirred for 1 h at room temperature. To the clear solution formed was added a solution of pyrimidine-2-thione (0.037 g, 0.30 mmol) and slow evaporation of this solution at room temperature gave colorless crystals of 7 (yield 0.08 g, 72%, mp 85–88 °C). Elemental analysis for Ag₉H₃₆N₄P₂S₂O₆ (775.59): calcd C 63.43; H 4.77; N 4.99, S 3.80%; found: C 63.08; H 5.15; N 4.93, S 3.76%. Complex 9 was prepared similarly.

X-ray crystallography
Molecular structures of complexes 2, 3, 5–8 which formed good quality crystals have been solved by X-ray Crystallography; the quality of other complexes has been poor. The crystal of a complex was mounted on a polymer loop and data were measured on a Rigaku-Oxford Diffraction (2, 5–8), Agilent, Eos, Gemini diffraction meter (3) at 173(2) (3, 8) and 293(2) (2, 5–7) K. The diffractometer was equipped with a graphite monochromator with Mo–Kz radiation (λ = 0.71073 Å; 3, 8), or Cu-Kz radiation (λ = 1.54178 Å; 2, 5–7). The data collection and cell refinement were processed with CrysAlisPro while data reduction was processed with CrysAlisRED (2, 3, 5–8). The structures were solved by the direct methods and refined using full-matrix least-squares techniques based on F² using ShelXL-2008, 2015. Table 4 contains crystal data.

Antimicrobial studies
Test organisms and inoculum preparation. The reference strains of bacteria and yeast were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Reference strains included Gram positive bacteria: Staphylococcus aureus (MTCC 740), methicillin resistant Staphylococcus aureus (MRSA) Staphylococcus epidermidis (MTCC 435), Enterococcus faecalis (MTCC 439), Gram negative bacteria Shigella flexneri (MTCC 1457), Escherichia coli (MTCC 119) and a yeast Candida albicans (MTCC 227). A loopful of bacterial and yeast cells were inoculated into 5 mL of their respective broth and incubated at 37 °C and 25 °C, respectively for 4 h. This was used as inoculum after adjusting the turbidity as per the McFarland standard. This turbidity is equivalent to approximately (1 to 2) × 10⁸ colony forming units per mL (CFU per mL). The inoculums thus prepared were used for further testing.

Minimum inhibitory concentration (MIC)
Minimum inhibitory concentration of the selected coordination compound dissolved in DMSO was worked out by the agar dilution method for their antimicrobial activity against the sensitive microorganisms. From the stock solution, the concentration of a complex in the range (0.001–2 mg mL⁻¹) was mixed with a Muller Hinton agar medium and then poured on plates. These plates were then inoculated with 0.1 mL of the activated bacterial and yeast strains by streaking with a sterile swab. The plates were incubated at 37 °C for bacteria and 25 °C for yeast for 24 h each. The minimum concentration of the extract causing inhibition of the visible growth was taken as MIC. The results were compared with that of DMSO as a control.

MTT toxicity assay
In order to check the level of cellular toxicity of the test compounds, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was performed as follows. Ten milliliters of sheep blood was taken into an injection syringe containing 3 mL of Alsever’s solution (anticoagulant) and transferred to sterile centrifuge tube. The blood was centrifuged at 16 000 rpm at room temperature (25 °C) for 20 min so as to separate the plasma from the cells. The supernatant was
discarded and 6 mL phosphate buffer saline (PBS) was added and it was again centrifuged. The blood cells were washed thrice with PBS by centrifugation and the pellet was re-suspended in 6 mL of PBS. Various dilutions of these cells were prepared using PBS and counted with the help of a hemocytometer under a light microscope so as to obtain cells equivalent to $1 \times 10^5$ cells per mL. One hundred microliters (100 μL) of this diluted suspension was added in each well and incubated at 37 °C for overnight. The supernatant was removed carefully and 200 μL of the sample solution (contains 10 mg mL$^{-1}$) was added and incubated further for 24 h. The supernatant was removed again and 20 μL MTT solution (5 mg mL$^{-1}$) was added to each well and incubated further for 3.5 h at 37 °C on an orbital shaker at 60 rpm. After incubation, the supernatant was removed without disturbing the cells and 50 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 590 nm using an automated microplate reader (Biorad 680-XR, Japan). The wells with untreated cells served as a control. Reduction of MTT can only occur in metabolically active cells, as MTT is converted into formazan crystals and hence the absorbance directly represents the viability of the cells (% viability = (optical density (OD) of treated/OD of control) × 100). If the percent viability of the blood cells is quite high, then the compounds are non-cytotoxic in nature.

Antitumor studies

To determine in vitro cytotoxicity of silver(i) complexes of heterocyclic thioureas, MTT assay MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide],$^{72}$ was performed on a human osteosarcoma cell line, MG63. The cell line was procured from National Cell Center, Pune, India and was grown and matured in Dulbecco’s Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) in tissue culture flask in the incubator supplied with 5% CO$_2$ at 37 °C and in 90% relative humidity provided by the growth medium. These cells were then collected in the confluent phase by treating with Trypsin-Hank’s Balanced Salt Solution (HBSS). The cell suspension was centrifuged at 1500 rpm for 15 min. The supernatant was removed and 5 mL of medium (DMEM) was added to the cell pellet. The cell number was counted using hemocytometer under a light microscope and the cell density was made to 40 000 cell per mL (in DMEM) in the cell suspension.

The 100 μL of cell suspension was added to each well of the 96-well plate and the plate was kept in the CO$_2$ incubator with 5% CO$_2$, at 37 °C for 24 h. The culture was further incubated for 24 h in the presence of 100 μL of each of silver(i) complex of varying concentration (100, 50, 25, 12.5 and 6.25 μM) diluted in DMEM. The supernatant was removed from each well and 100 μL of MTT (0.5 mg mL$^{-1}$ in DMEM) was added to each well, and further incubated at 37 °C for 4 h. After incubation, the supernatant was removed and the dark purple colored MTT formazan crystals were formed at the bottom of each of the well. The MTT formazan crystals thus formed were resuspended in 100 μL of dimethyl sulfoxide and the 96-well plate was shaken in a Lab-system Multiskan EX ELISA reader for 3 minute to dissolve the formazan crystals. The absorbance of the purple colored solution thus formed was then measured at 570 nm. The wells with untreated cells served as control. Reduction of MTT can only occur in metabolically active cells, as MTT is converted into formazan crystals and hence the absorbance directly represents the viability of the cells (% viability = (optical density (OD) of treated/OD of control) × 100). The IC$_{50}$ values (50% inhibitory concentration), which is defined as the concentration of complex that reduced the number of living cancerous cells by 50%, were calculated from three independent experiments by generating an equation of logarithmic trend line of percentage cell viability against concentration of compounds in Microsoft excel.

Conflicts of interest

There are no conflicts to declare.

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