

Synthesis, characterisation and antimicrobial activity 1-aminopyrimidine-2(1*H*)-thione and its Co(II), Ni(II), Pd(II) and Pt(II) complexes

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Metal complexes of 1-aminopyrimidine-2(1*H*)-thione were prepared from acetate salts of Co(II), Ni(II), Pd(II) and Pt(II) in methanol. Electronic spectral data and magnetic moment measurements suggest mononuclear square planar geometrical structures for the metal complexes. The ligand and all the metal complexes were evaluated for their antimicrobial activity. The newly synthesised ligand and the Co(II) complex showed moderate activity against all tested bacteria and yeast strains.

Keywords: 1-aminopyrimidine-2(1*H*)-thione, metal complexes, antimicrobial activity

Pyrimidine is the parent heterocycle of an important group of compounds that occur in living systems.¹ There has been considerable attention in metal (II) complexes of polydentate Schiff base ligands of *N*-aminopyrimidine type, due to their structural richness, electrochemical properties as well as their being a potential model for a number of important biological systems.² Heterocyclic thiones form the backbone of more complicated organic ligands using other sites for attachment to metals. Thioketo groups like 1,3-dithiole-2-thiones form a vast and expanding field of research with technological applications.³

In view of the biological significance and diverse coordinating behaviour of pyrimidines as well as the semiconducting properties of some first row transition elements which have been found to depend on the structure of the complex, we prepared and studied some of these compounds.

We now describe the synthesis of novel metal (II) complexes of bidentate ligand containing a ring of the mercapto pyrimidine. Spectral and magnetic studies have been used to characterise the structure of the complexes.

Results and discussion

1-Aminopyrimidine-2(1*H*)-thione (N-APTH) was prepared according to the literature method by a two-step process.⁴ The ligand and its complexes are very stable at room temperature in the solid state. The ligand is soluble in common organic solvents, but its metal complexes are generally only soluble in DMF and DMSO. The elemental analytical data of the complexes reveal a metal:ligand stoichiometry of 1:2 corresponding to the square planar geometry of [M(N-APT)₂] $\cdot n$ H₂O (Fig. 1). These analytical data are in good agreement with the

proposed stoichiometry of the complexes. The molar conductivities of compounds 1–3 in DMF at 25 °C are in the range of 3.16–16.11 $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$, indicating non-electrolytes.⁵

IR spectra of the ligand show characteristic bands at 3241 cm^{-1} $\nu(\text{NH}_2)$, 3059 cm^{-1} $\nu(\text{C-H pyrimidine ring})$, 1648 cm^{-1} $\nu(\text{C=O benzoyl})$, 1600 ($\text{C=N}_{\text{pyrimidine}}$) 1104, 713 $\nu(\text{C=S})$ vibrations.^{6,7} IR spectra of the complexes were compared with that of the free ligand to show changes during complexation. The Co(II), Ni(II), Pd(II) and Pt(II) complexes of the ligand 3241 cm^{-1} for $\nu(\text{NH})$ primer amine asymmetric-symmetric stretching vibration bands vanished and the sec-amine peak was observed at 3337–3423 cm^{-1} . Also, the $\nu(\text{C=S})$ vibration at 1104, 713 cm^{-1} in the free ligand shifts to lower frequency at 1092, 698 cm^{-1} after complexation due to coordination with sulfur atom of thione group to the metal ion.^{6–8} This situation indicated coordination of sulfur and nitrogen to metal(II) ion.⁹ Thus, the metal–ligand bond formed over the NH₂ group, which lost one proton of ligand.¹⁰ The low frequency region of the spectra revealed the presence of two new medium intensity bands at 436 cm^{-1} and 566 cm^{-1} due to $\nu\text{M-S}$ and $\nu\text{M-N}$ vibrations.¹⁰

Electronic spectra were recorded in DMF. In the ligand, the band at 363 nm is attributed to $\pi-\pi^*$ of the azomethine. Bands at 316 and 268 nm are associated with phenyl and pyrimidine $\pi-\pi^*$ transitions. In spectra of the complex, the $\pi-\pi^*$ of the azomethine shifted to 370 nm, indicating that the azomethine nitrogen is involved in coordination. UV-vis spectra of Ni(II) complex shows three d–d bands at 562, 525 and 461 nm. These bands can be assigned to $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}$ (F) and $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}$ (F), respectively. Transitions are characteristic of square planar configurations for Co(II) and Ni(II) complexes.^{11,12} The Co(II) complex shows a magnetic moment of 2.01 B.M. which is in good agreement with spin only value. This is further evidenced by the electronic spectral data, which shows absorption in the region 590 nm corresponding to $^1\text{A}_{1g} \rightarrow ^1\text{B}_{1g}$ transitions which also supports square planar geometry.^{12,13} Palladium(II) and platinum(II) are a d^8 system and three spin allowed singlet–singlet d–d transitions are predicted.¹⁴ The ground state is $^1\text{A}_{1g}$ and excited states corresponding to the above transitions are $^1\text{A}_{2g}$, $^1\text{B}_{1g}$ and $^1\text{E}_g$ in order of increasing energy. Three d–d bands are observed in the region 488–585, 469–393 and 388–384 nm, respectively. These bands are attributed to $^1\text{A}_{1g} \rightarrow ^1\text{A}_{2g}$, $^1\text{A}_{1g} \rightarrow ^1\text{B}_{1g}$ and to $^1\text{A}_{1g} \rightarrow ^1\text{E}_g$ transitions, respectively. The electronic spectra of these complexes indicate the square planar geometry and the values obtained correspond to those reported earlier for the square planar complexes.¹⁵

DMSO-*d*₆ was used as a solvent to measure the ¹H NMR spectra of Pd(II) and Pt(II) complexes. ¹H NMR spectra of the Pd(II) and Pt(II) complexes show a signal corresponding to the primary amine at about 2.50 ppm. Sharp singlets at 8.92 ppm and 8.73 ppm are due to C6(H)–pyrimidine proton; the phenyl

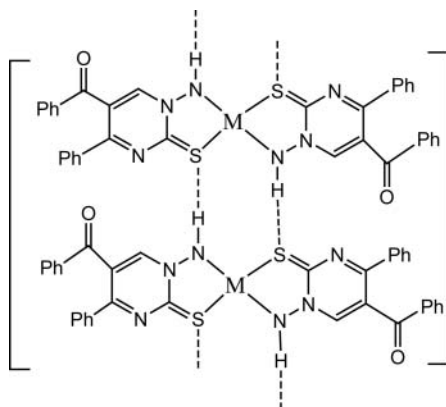


Fig. 1 Proposed structure of all the complexes.

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multiplets successively were between δ 7.22–7.92 ppm and 7.11–7.93 ppm.^{13,14}

The LC-API-ES mass spectrum of $[\text{Co}(\text{NAPT})_2]^+$, $[\text{Ni}(\text{N-APT})_2]^+$, $[\text{Pd}(\text{N-APT})_2+2]^+$, $[\text{Pt}(\text{N-APT})_2+1]^+$ showed a molecular ion peak M^+ at m/z 691.0, 670.3, 670.3, 757.3 and 826.8, respectively that is equivalent to its molecular weight. All the compounds are consistent with the molecular ion fragment and support the proposed structure of the complexes.

The TGA data agree with the formula suggested from elemental analyses. The thermal stabilities were investigated using differential thermal and thermal gravitational analysis (DTA/TGA) at a heating rate of $10^\circ\text{C min}^{-1}$ in N_2 from 20 to 1000°C . Mass losses correspond to H_2O , Ph-CO- and the other organic moieties in the first, second, third, fourth, and fifth stages of decomposition. The Co(II), Pd(II) and Pt(II) complexes suffered loss of H_2O in the first stage, $37\text{--}130^\circ\text{C}$, and the ligands gradually decomposed from 220 to 580°C . The Co(II), Pt(II) and Pd(II) complexes contain 1, 1 and 2 moles of water of crystallisation per complex molecule, respectively. The complexes had quite higher carbon residue with CoO, NiO, Pd and Pt when they were heated to 1000°C .^{13,14} The thermal stability of the complexes of N-PTH was found to follow the order $\text{Pd(II)} < \text{Pt(II)} \approx \text{Co(II)} < \text{Ni(II)}$. The thermogravimetric curve of the Co(II) complex is shown in Fig. 2.

The ligand and its metal complexes [Co(II), Ni(II), Pd(II) and Pt(II)] were screened for antibacterial activity against *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 25923,

Bacillus cereus ATCC 7064, *Micrococcus luteus* ATCC 9345 and *Escherichia coli* ATCC 4230 and for antifungal activity against *Candida albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 using broth microdilution procedures.^{15,16} Ampicillin trihydrate for bacteria and fluconazole for yeast were used as the reference drugs. The results of the antimicrobial activity of the ligand and its metal complexes against all tested bacterial and fungal strains are shown in Tables 1 and 2. All compounds inhibited the growth of bacteria (Gram-negative and Gram-positive) with MIC values in the range of $40\text{--}160\text{ }\mu\text{g mL}^{-1}$ as well as exhibited antifungal activity with MICs between 40 and $80\text{ }\mu\text{g mL}^{-1}$. The Co(II) complex was found to possess an effective and selective antibacterial activity against one Gram-negative bacterium (*E. coli* ATCC 4230) and other Gram-positive bacteria (*S. aureus* ATCC 6538, *S. aureus* ATCC 25923 and *M. luteus* ATCC 9345) with MIC values in range of $40\text{ }\mu\text{g mL}^{-1}$. On the other hand, other compounds had moderate antibacterial activity against all Gram positive and Gram-negative bacteria with MICs in the range of $80\text{ }\mu\text{g mL}^{-1}$ except for the Ni(II) complex which was $160\text{ }\mu\text{g mL}^{-1}$. Moreover, our findings indicated that all prepared compounds had similar antibacterial efficacy against Gram-positive and Gram-negative bacteria. Table 2 summarises the antifungal activities of the free ligand and the complexes against three yeast strains (*C. albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019). According to antifungal studies, the ligand and Co(II) complexes compounds displayed good anti-yeast efficacy against

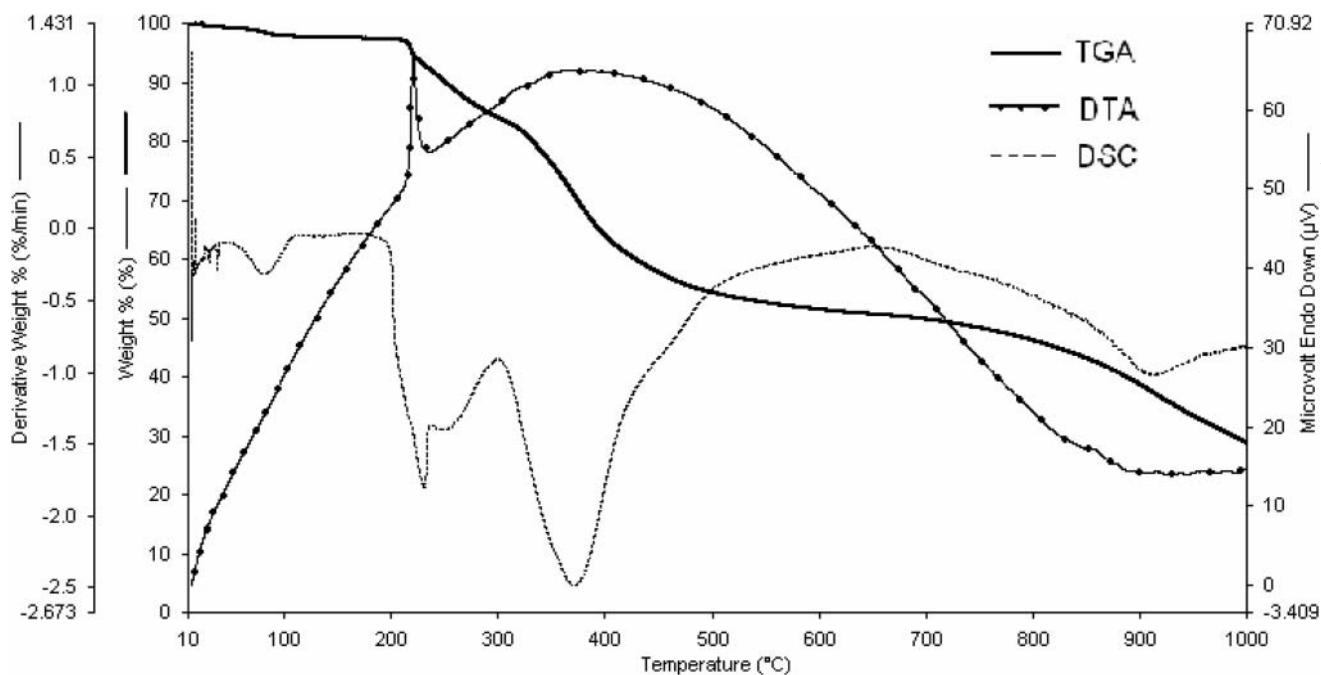


Fig. 2 Thermogravimetric curves of the Pd(II) complex.

Table 1 MICs^a of the ligand (N-APTH) and its metal complexes [Co(II), Ni(II), Pd(II), Pt(II)] against Gram-negative and Gram-positive bacterial strains

Compounds	<i>Bacillus cereus</i> ATCC 7064	<i>Staphylococcus aureus</i> ATCC 6538	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 4230	<i>Micrococcus luteus</i> ATCC 9345
N-APTH	80	80	80	80	40
1	80	40	40	40	40
2	160	160	160	160	160
3	80	80	80	80	80
4	80	80	80	80	80
Ampicillin	5	5	10	20	10

^aThe MICs values were determined as $\mu\text{g mL}^{-1}$ active compounds in medium.

Table 2 MICs^a of the ligand (*N*-APTH) and its metal complexes [Co(II), Ni(II), Pd(II), Pt(II)] against fungal strains

Compounds	<i>Candida albicans</i> ATCC 14053	<i>Candida parapsilosis</i> ATCC 22019	<i>Candida krusei</i> ATCC 6258
<i>N</i>-APTH	40	40	40
1	80	80	80
2	80	80	80
3	80	80	80
4	80	80	80
Fluconazole	5	5	10

^a The MICs values were determined as $\mu\text{g mL}^{-1}$ active compounds in medium.

ranging between 40 and 80 $\mu\text{g mL}^{-1}$. Finally, the reason for the good antimicrobial efficacy could be related to the inhibition of several structural enzymes which play a key role in vital metabolic pathways of the microorganisms.

Experimental

Elemental analyses (C, H, N, S) were performed by using a Leco CHNS model 932 elemental analyser. The IR spectra were obtained using KBr discs (4000–400) cm^{-1} on a Bio-Rad-Win-IR spectrophotometer. The electronic spectra in the 200–900 nm range were obtained in DMF on a Unicam UV2-100 UV-Vis spectrophotometer. Magnetic measurements were carried out by the Gouy method using $\text{Hg}[\text{Co}(\text{SCN})_4]$ as calibrant. Molar conductance of the ligand and their transition metal complexes were determined in DMF at room temperature by using a Jenway model 4070 Conductivity meter. The ^1H NMR, Pt and Pd complexes were recorded with a Bruker 300 MHz Ultrashield TM NMR instrument. LC/MS-API-ES mass spectra were recorded using an Agilent model 1100 MSD Mass Spectrophotometer. Thermal data were obtained by using Perkin-Elmer Diamond Thermal Analysis. DTA/TGA measurements were made between 20 and 1000 $^{\circ}\text{C}$ in N_2 , 10 $^{\circ}\text{C min}^{-1}$.

Synthesis of the ligand

1-Amino-5-benzoyl-4-phenylpyrimidine-2(1*H*)-thione was prepared according to the literature method by a two-step process.⁴

An equimolar mixture of **1** and the corresponding thiosemicarbazone **2** was heated to 80 $^{\circ}\text{C}$ in dry toluene for 1 h. After 12 h at temperature, a yellow precipitate was separated out by filtration. Product **3** was recrystallised from *n*-butanol and dried in a vacuum desiccator. The yield was 0.302 g (34%), m.p. 226 $^{\circ}\text{C}$. (Scheme 1a). Compound **3** (1 g) was dissolved in 25 mL of concentrated hydrochloric acid and 200 mL of water was added to give a precipitate of **4** (Scheme 1b.) The precipitate was filtered off, recrystallised from ethanol and dried in a vacuum desiccator. The yield was 0.52 g (70%), m.p. 195 $^{\circ}\text{C}$. Selected IR data (KBr, $\nu \text{ cm}^{-1}$): 3240 $\nu(\text{NH}_2)$, 3059 (C–H_{pyrimidine}), 1648 $\nu(\text{C=O})$, 1600 (C=N_{pyrimidine}) 1104, 713 $\nu(\text{C=S})$.

Synthesis of the complexes (**1–4**)

N-APTH 0.307 g (1.00 mmol) was dissolved in 25 mL of chloroform/25 mL methanol, and a solution of (0.5 mmol) $\text{Co}(\text{AcO})_2 \cdot 4\text{H}_2\text{O}$ (0.125 g), $\text{Ni}(\text{AcO})_2 \cdot 4\text{H}_2\text{O}$ (0.124 g) $\text{Pd}(\text{AcO})_2$ (0.112 g), and PtCl_2

(0.133 g) methanol was added dropwise with continuous stirring. The mixture was stirred further for 1 h at 60 $^{\circ}\text{C}$. The precipitated solid was then filtered off, washed with diethyl ether, followed by cold methanol and dried in a vacuum desiccator.

$[\text{Co}(\text{N-APT})_2] \cdot \text{H}_2\text{O}$ (**1**): Yield was 305 mg (88%), m.p. 230 $^{\circ}\text{C}$. Anal. Calcd for $\text{C}_{34}\text{H}_{26}\text{CoN}_6\text{O}_3\text{S}_2$ (689.67): C, 59.21; H, 3.80; N, 12.19; S, 9.30. Found: C, 59.35; H, 3.79; N, 12.21; S, 9.93%. Selected IR data (KBr, $\nu \text{ cm}^{-1}$): 3337 $\nu(\text{NH})$, 3059 (C–H_{pyrimidine}), 1652 $\nu(\text{C=O})$, 1594 (C=N_{pyrimidine}) 1121, 740 $\nu(\text{C=S})$ 510–520 (M–O), 430–440 (M–N). UV-Vis [λ (nm), ϵ ($\text{M}^{-1}\text{cm}^{-1}$)]: 224, 274, 290, 308, 332, 361, 379, 395, 413, 590. μ_{eff} , 2.01 BM, Δo ($\text{S cm}^2 \text{ mol}^{-1}$) 16.11. API-ES: m/z 691.0 [$^{59}\text{Co}(\text{N-APT})_2$]⁺.

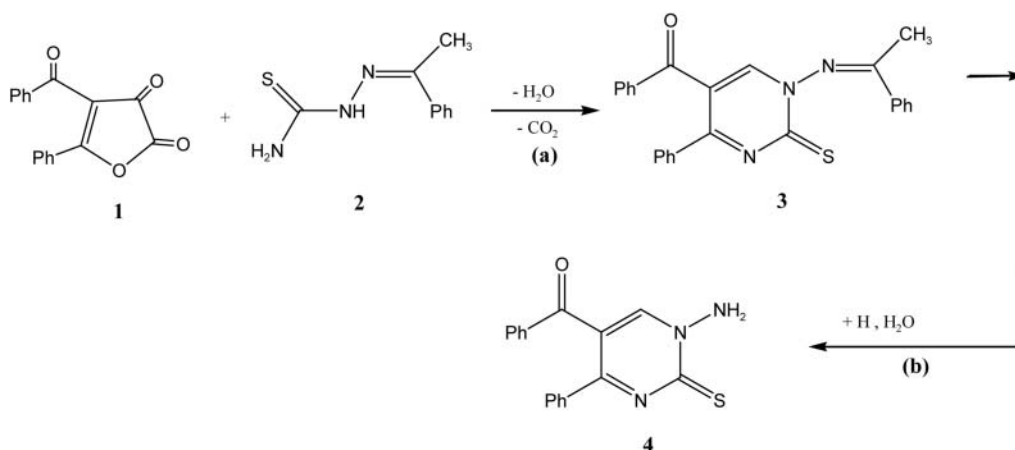
$[\text{Ni}(\text{N-APT})_2]$ (**2**): Yield was 298 mg (88%), m.p. 241 $^{\circ}\text{C}$. Anal. Calcd for $\text{C}_{34}\text{H}_{24}\text{NiN}_6\text{O}_2\text{S}_2$ (671): C, 60.82; H, 3.60; N, 12.52; S, 9.99. Found: C, 60.51; H, 3.74; N, 12.50; S, 9.55%. Selected IR data, ν (cm^{-1}): 3343 $\nu(\text{NH})$, 3059 (C–H_{pyrimidine}), 1652 $\nu(\text{C=O})$, 1593 (C=N_{pyrimidine}) 1116, 738 $\nu(\text{C=S})$ 510–520 (M–O), 430–440 (M–N). UV-Vis [λ (nm), ϵ ($\text{M}^{-1}\text{cm}^{-1}$)]: 253, 280, 313, 337, 375, 397, 411, 461, 525, 562. μ_{eff} , Dia, Δo ($\text{S cm}^2 \text{ mol}^{-1}$) 3.16. API-ES: m/z 670.3 [$^{58}\text{Ni}(\text{N-APT})_2$]⁺.

$[\text{Pd}(\text{N-APT})_2] \cdot 2\text{H}_2\text{O}$ (**3**): Yield was 355 mg (84%), m.p. 251 $^{\circ}\text{C}$. Anal. Calcd for $\text{C}_{34}\text{H}_{28}\text{N}_6\text{O}_4\text{PdS}_2$ (756.06) C, 54.08; H, 3.74; N, 11.13; S, 8.49. Found: C, 54.59; H, 3.51; N, 10.79; S, 8.51%. Selected IR data, ν (cm^{-1}): 3421 $\nu(\text{NH})$, 3059 (C–H_{pyrimidine}), 1665 $\nu(\text{C=O})$, 1596 (C=N_{pyrimidine}) 1144, 741 $\nu(\text{C=S})$ 510–520 (M–O), 430–440 (M–N). UV-Vis [λ (nm), ϵ ($\text{M}^{-1}\text{cm}^{-1}$)]: 258, 273, 287, 297, 305, 323, 353, 376, 388, 469, 488. μ_{eff} , Dia, Δo ($\text{S cm}^2 \text{ mol}^{-1}$) 6.12. API-ES: m/z 757.3 [$^{108}\text{Pd}(\text{N-APT})_2 + 1$].

$[\text{Pt}(\text{N-APT})_2] \cdot \text{H}_2\text{O}$ (**4**): Yield was 225 mg (49%), m.p. 216 $^{\circ}\text{C}$. Anal. Calcd for $\text{C}_{34}\text{H}_{26}\text{N}_6\text{O}_3\text{PtS}_2$ (825.12 g/mol): C, 49.45; H, 3.17; N, 10.18; S, 7.77. Found: C, 50.13; H, 3.15; N, 10.33; S, 8.17%. Selected IR data, ν (cm^{-1}): 3423 $\nu(\text{NH})$, 3058 (C–H_{pyrimidine}), 1664 $\nu(\text{C=O})$, 1597 (C=N_{pyrimidine}) 1145, 742 $\nu(\text{C=S})$ 510–520 (M–O), 430–440 (M–N). UV-Vis [λ (nm), ϵ ($\text{M}^{-1}\text{cm}^{-1}$)]: 271, 285, 290, 294, 351, 373, 384, 393, 585. μ_{eff} , Dia, Δo ($\text{S cm}^2 \text{ mol}^{-1}$) 14.4. API-ES: m/z 826.8 [$^{192}\text{Pt}(\text{N-APT})_2 + 1$].

Antibacterial assay

Newly synthesised compounds were screened for antibacterial activity against four Gram-positive (*S. aureus* ATCC 6538, *S. aureus* ATCC 25923, *B. cereus* ATCC 7064 and *M. luteus* ATCC 9345) and one Gram-negative (*E. coli* ATCC 4230) bacteria as described by the

**Scheme 1**

guidelines in NCCLS approved standard document M7-A4 using the microdilution broth procedure.¹⁵ Ampicillin trihydrate was used as the reference antibacterial agent. Solutions of the compounds and reference drug were dissolved in DMSO at a concentration of 2560 $\mu\text{g mL}^{-1}$. Two-fold dilution of compounds and reference drug were prepared (1280, 640, 320, 160, 80, 40, 20, 10, 5 > $\mu\text{g mL}^{-1}$). Antibacterial activities of the newly synthesised compounds were determined in the Mueller–Hinton broth (Difco) medium at pH 7.2 with an inoculum of $(1-2) \times 10^3$ cells mL^{-1} by the spectrophotometric method and an aliquot of 100 μL was added to each tube of serial dilution. The compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37 °C for 18 h at 150 rpm. The minimum inhibitory concentrations (MICs) of each compound were recorded as lowest concentration of each compound in the tubes with no growth (*i.e.* no turbidity) of inoculated bacteria.

Antifungal assay

The antifungal activities of new synthesised chemical compounds were tested against three yeast (*C. albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) strains according to the guidelines in NCCLS approved standard document M27-A2 using the microdilution broth procedure.¹⁶ Fluconazole was used as the reference antifungal agent. Solution of the test compounds and reference drug were dissolved in DMSO at a concentration of 2560 $\mu\text{g mL}^{-1}$. The two-fold dilution of the compounds and reference drug were prepared (1280, 640, 320, 160, 80, 40, 20, 10, 5 > $\mu\text{g mL}^{-1}$). Antifungal activities of the yeast strains were performed in RPMI 1640 Medium (Sigma) which had been buffered to pH 7.0 with 0.165 M morpholinopropanesulfonic acid (Sigma), as outlined in document M27-A. The stock yeast inoculum suspensions were adjusted to a concentration of $(0.5-2.5) \times 10^3$ cells mL^{-1} by spectrophotometric method and 100 μL aliquot was added to each tube of dilution. The chemical compound-broth medium serial tube dilutions inoculated with yeast were incubated on a rotary shaker at 37 °C for 18 h at 150 rpm. The minimum inhibitory concentrations (MICs) of each chemical compounds were recorded as lowest concentration of each chemical compounds in the tubes with no growth (*i.e.* no turbidity) of inoculated yeast.

Conclusion

The synthesis and structure of new heterocyclic ligand and its metal complexes in 1:2 metal:ligand ratios have been described. The ligand is a bidentate or tridentate chelating agent coordinating through a deprotonated amine group and sulfur of pyrimidine thione group. The analytical data, electronic spectra, magnetic susceptibility, IR, NMR, and API-ES mass spectral data reveal mononuclear square-planar configuration of Co(II) and Ni(II), Pd(II) and Pt(II) complexes. Single crystals of the compounds could not be isolated and definite structures

cannot be described. However, the spectroscopic and magnetic data enable us to predict possible structures. The antibacterial activity results evidently show that all the complexes have moderate activity against Gram-positive bacteria. They have weak activity against Gram-negative bacteria, except the ligand and Co(II) complex which has moderate activity against *E. coli*.

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