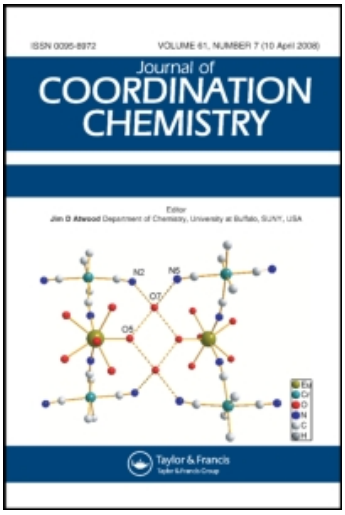


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# Synthesis, coordination behavior, and investigations of pharmacological effects of some transition metal complexes with isoniazid Schiff bases

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# Synthesis, coordination behavior, and investigations of pharmacological effects of some transition metal complexes with isoniazid Schiff bases

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Two isoniazid Schiff bases, N-isonicotinamido-2-furanketimine (INH-$F^1$) and N-isonicotinamido-5-methyl-2-furanketimine (INH-$F^2$), possessing potential N and O coordination sites have been prepared by the reaction of isoniazid with 2-acetylfuran and 2-acetyl-5-methylfuran, respectively. Complexes of Pd(II) and Pt(II) have been prepared and characterized by elemental analyses, melting point determinations and electronic, infrared, $^1$H NMR, $^{13}$C NMR spectral studies, and X-ray powder diffraction studies. In all the complexes, the monobasic bidentate nature of the ligand is evident. Antibacterial and antifungal studies of these compounds against various pathogenic bacterial and fungal strains have been carried out. Both the ligands and their metal chelates were active against all the microbial strains investigated. However, the chelates were found to be more active than the ligands. The antimycobacterial activity of the ligands and their metal complexes has been evaluated against *Mycobacterium smegmatis*, which showed clear enhancement in this activity upon metal complexation with Schiff bases.

*Keywords*: Isoniazid Schiff bases; Pd(II) and Pt(II) complexes; Antifungal; Antibacterial and antimycobacterial activity

## 1. Introduction

Heterocyclic compounds are widely distributed in nature and are essential for many biochemical processes. The past few decades have seen the introduction of a number of pharmaceutical compounds which contain five, six, and seven-membered rings such as piperazines, piperidines, imidazoles, benzodiazepines, and other heterocycles containing nitrogen, sulfur, and oxygen [1]. Systems of this kind play a significant role in many biological processes due to their metal-coordinating ability [2]. Bioinorganic compounds and biomimetics, containing a C=N bond, are widely represented [3]. Schiff bases have been intensively investigated for their strong coordination capability and diverse biological activities [4–7]. The tuberculostatic activity of isonicotinic acid hydrazide and its aroylhydrazones containing azomethine nitrogen is attributed to their ability to form stable complexes with d- and f-block metal ions [8–10]. Interest in the study of hydrazones has been growing because of their antimicrobial, antituberculosis,

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and antitumour activity [11–14]. Isoniazid Schiff bases have been found to show better anticancer and antitubercular activity than isoniazid [15, 16].

The remarkable biological activity of acid hydrazides R−CO−NH−$NH_2$ as well as their corresponding aroylhydrazones R−CO−NH−N=CH−R, and the dependence of their mode of chelation with transition metal ions present in the living system have been of great importance [17–20]. Enzymatic acetylation of the antitubercular isoniazid by N-acetyltransferase represents a major metabolic pathway for isoniazid in humans. Acetylation greatly reduces the therapeutic activity of the drug, resulting in underdosing, decreased bioavailability, and acquired isoniazid resistance. Chemical modification of the hydrazine unit of isoniazid with a functional group that blocks acetylation, while maintaining strong antimycobacterial action, has the potential to improve clinical outcomes and to reduce the emergence in patients of acquired isoniazid resistance. Isoniazid Schiff bases and their metal complexes exhibit better antimicrobial activity than isonicotinic acid hydrazide [21, 22].

Considering the constant emergence of antibiotic resistance to clinically used compounds, it is critical to develop new antibiotic classes, which eventually would target the lipoid layer of the organisms and other aspects of the pathogen life cycle. Interest in platinum and palladium complexes is still rapidly growing because of the attempts to find complexes with greater potency and less toxicity than the existing clinical drugs [23, 24]. A careful literature survey indicated that very little work is known about Pd(II) and Pt(II) complexes of hydrazones having an isoniazid moiety. In the quest for biologically more potent antibacterial and antitubercular compounds, we envisioned two isoniazid Schiff bases, N-isonicotinamido-2-furanketimine (INH-$F^1$) and N-isonicotinamido-5-methyl-2-furanketimine (INH-$F^2$) and their Pd(II) and Pt(II) complexes. The results of preparation, spectroscopic investigations, antibacterial, antifungal, and anti-mycobacterial activities of the synthesized ligands and their metal chelates are discussed in this article.



## 2. Experimental

### 2.1. *Chemicals*

Palladium and platinum salts, $PdCl_2$ and $PtCl_2$ as well as 2-acetyl-5-methylfuran were purchased from Alfa Aesar and isoniazid was purchased from SD Fine Chemicals Ltd. (Mumbai, India). Solvents of AR grade were distilled from appropriate drying agents prior to use.

### 2.2. *Physical measurements and analyses*

Molecular weights of the synthesized ligands and their metal complexes were determined by the Rast Camphor method. Chlorine was estimated by Volhard's method and nitrogen was estimated by the Kjeldahl's method. Carbon and hydrogen analyses were performed at the CDRI, Lucknow. Pd(II) and Pt(II) were estimated gravimetrically. Electronic spectra were recorded on a Varian–Cary/5E spectrophotometer at SAIF, IIT, Madras, Chennai. IR spectra of the ligands and their complexes were recorded with the help of a Nicolet Magna FTIR-550

spectrophotometer from 4000–200 $cm^{-1}$ in KBr pellets. $^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer in DMSO-$d_6$ using TMS as the internal standard. X-ray powder diffractograms of the compounds were obtained on a Philip Model PW 1840 automatic diffractogram using Fe (K$\alpha$) target with Mg filter. The wavelength used was 1.93604 Å.

### 2.3. *Synthesis of aroylhydrazones*

Both of the ligands, N-isonicotinamido-2-furanketimine (INH-$F^1$) and N-isonicotinamido-5-methyl-2-furanketimine (INH-$F^2$), were prepared as reported earlier [25] and their physical properties and analytical data are given in table 1. The synthetic route of the ligands is shown in figure 1.

Table 1. Analytical data and physical properties of the ligands and their complexes.

| Compound | Color and m.p. (°C) | Yield (%) | Found (Calcd) (%) C | H | N | Cl | M | Molecular weight Found (Calcd) |
|---|---|---|---|---|---|---|---|---|
| (**1**) INH-$F^1$ | Brown 232–235 | 70 | 57.02 (57.67) | 3.99 (4.83) | 19.75 (18.33) | – | – | 205.34 (229.23) |
| (**2**) INH-$F^2$ | Yellow 239–242 | 76 | 58.99 (59.25) | 5.03 (5.38) | 16.99 (17.29) | – | – | 298.35 (243.26) |
| (**3**) $[Pd(INH\text{-}F^1)_2]Cl_2$ | Brown 165–167 | 72 | 41.04 (41.56) | 2.98 (3.48) | 12.97 (13.21) | 11.02 (11.15) | 16.24 (16.73) | 599.01 (635.77) |
| (**4**) $[Pd(IN\text{-}F^1)_2]$ | Yellow 172–174 | 67 | 46.12 (46.94) | 3.01 (3.58) | 14.68 (14.93) | – | 18.36 (18.90) | 530.08 (562.85) |
| $[Pd(INH\text{-}F^2)_2]Cl_2$ | Yellow 244–246 | 68 | 43.03 (43.42) | 3.32 (3.94) | 11.99 (12.65) | 9.99 (10.68) | 26.98 (26.70) | 624.38 (663.83) |
| $[Pd(IN\text{-}F^2)_2]$ | Green 183–185 | 72 | 48.09 (48.78) | 3.97 (4.09) | 13.99 (14.22) | – | 29.95 (30.00) | 549.65 (590.91) |
| $[Pt(INH\text{-}F^1)_2]Cl_2$ | Brown 198–200(d) | 64 | 36.01 (36.47) | 2.95 (3.06) | 10.89 (11.60) | 9.10 (9.78) | 26.89 (26.92) | 685.29 (724.45) |
| $[Pt(IN\text{-}F^1)_2]$ | Coffee 205–208(d) | 66 | 40.00 (40.55) | 2.99 (3.09) | 11.78 (12.89) | – | 29.84 (29.94) | 612.12 (651.53) |
| (**5**) $[Pt(INH\text{-}F^2)_2]Cl_2$ | Brown 202–205(d) | 74 | 37.98 (38.30) | 3.02 (3.48) | 10.96 (11.16) | 8.98 (9.42) | 25.20 (25.92) | 703.98 (752.52) |
| (**6**) $[Pt(IN\text{-}F^2)_2]$ | Brown 164–166(d) | 70 | 42.02 (42.41) | 3.10 (3.55) | 11.98 (12.36) | – | 28.10 (28.70) | 635.24 (679.60) |

d = Decomposition point. Compounds **1**–**6** were tested for their antimicobacterial activity.

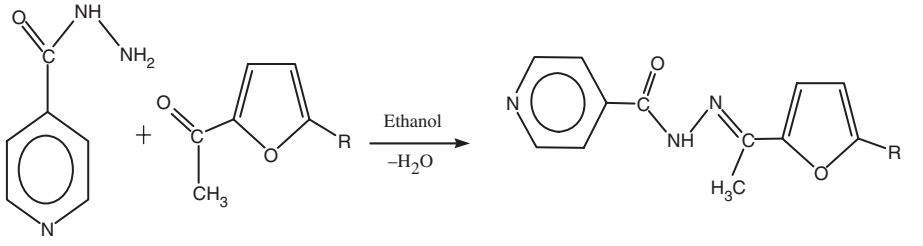



Figure 1. Synthetic route of the ligands, where R = H(INH-$F^1$) and $CH_3$(INH-$F^2$).

### 2.4. *Synthesis of the complexes*

For the preparation of $[Pd(IN\text{-}F)_2]$ complexes, methanol solution of $PdCl_2$ (0.42 g, 2.37 mmol) was mixed with methanol solution of INH-F[1] (1.08 g, 4.74 mmol) and INH-F[2] (1.15 g, 4.74 mmol) in 1 : 2 molar ratios, respectively. Aqueous ammonia was added drop wise to the reaction mixture until it was weakly alkaline (pH *ca* 8.0) and this reaction mixture was then heated under reflux for 1 h. On cooling, the complexes separated, were filtered, washed with methanol, and dried in vacuum. To obtain $[Pd(INH\text{-}F)_2]Cl_2$, methanol solution of $PdCl_2$ and the ligand in 1 : 2 molar ratio was stirred on a magnetic stirrer for 2–3 h in presence of a few drops of concentrated HCl.

For the preparation of platinum complexes, 1 : 1 water–ethanol solution of $PtCl_2$ (0.43 g, 1.61 mmol) was mixed with an ethanol solution of INH-F (0.74 g, 3.23 mmol) and INH-F[2] (0.78 g, 3.23 mmol) in 1 : 2 molar ratio, respectively. To obtain the $[Pt(IN\text{-}F)_2]$ aqueous ammonia was added dropwise to the reaction mixture until it was weakly alkaline (pH *ca* 8.0) and the reaction mixture was then heated under reflux for 1 h. $[Pt(INH\text{-}F)_2]Cl_2$ complexes have been synthesized by stirring the above reaction mixture on a magnetic stirrer for 2–3 h in the presence of a few drops of concentrated HCl in place of aqueous ammonia. The resulting products were washed with ethanol and dried in vacuum.



### 2.5. *Microbiology*

**2.5.1. Antifungal activity.** The ligands as well as their complexes were screened for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporum* using potato dextrose agar (potato 250 g + dextrose 20 g + agar 20 g) medium [26]. Solutions at 0.5, 1, and 1.5 mg $mL^{-1}$ of each compound in DMSO were prepared for testing against spore germination. A drop of the solution of each concentration was kept separately on glass slides. Conidia, the fungal reproducing spores (approximately 200), were lifted with the help of an inoculating needle and were mixed in every drop of each compound separately. Each treatment was replicated thrice and a parallel DMSO solvent control set was run concurrently on separate glass slides. All the slides were incubated in humid chambers at $25 \pm 2^{\circ}C$ for 24 h. Each slide was observed under the microscope for spore germination, and the percentage of germination was finally calculated. The results were also compared with a standard antifungal drug Flucanazole at the same concentrations.

**2.5.2. Antibacterial activity.** *In vitro* antibacterial screening is generally performed by disc diffusion methods for selection of compounds as therapeutic agents [26]. In this method, the activity of the test compounds is expressed by measuring the diameter of zone of inhibition. Generally, the more susceptible the organisms, the bigger the zone of inhibition. Each compound was dissolved in DMSO and solutions of concentration 2 and 1 mg $mL^{-1}$ were prepared separately. Paper discs of Whatman filter paper (No. 42) of uniform diameter (2 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in Petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *Escherichia coli* (−) and *Staphylococcus aureus* (+) bacteria separately. The Petri dishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of a common standard antibiotic Ampicillin was

also recorded using the same procedure as above at the same concentrations and using same the solvent. The % Activity Index for the complex was calculated by the formula:

$$\%\ \text{Activity Index} = \frac{\text{Zone of inhibilition by test compound (diamter)}}{\text{Zone of inhibilition by standard (diamter)}} \times 100$$

**2.5.3. Minimum inhibitory concentration (MIC) determination.** The MIC of the compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. The MIC was determined using the disc diffusion technique by preparing discs containing 0.1–1.0 mg mL$^{-1}$ of each compound against both bacteria and applying the protocol. All the compounds were more effective at 1.0 and 2.0 mg mL$^{-1}$ concentrations. Consequently, all the compounds were screened at these concentrations against both bacteria.

**2.5.4. *In vitro* antimycobacterial activity.** YT agar medium was prepared using 1% yeast extract, 2% trypton, 1.5% agar, 1% NaCl in 250 mL distilled water by maintaining the pH of the medium at 7 using 10% NaOH solution. This medium was then sterilized by autoclaving at 120°C for 15 min. After cooling to 50°C the medium was poured into 85 mm diameter Petri dishes (approx. 25 mL each) and setting aside at 37°C. After a few hours, Petri dishes were stored in the cold room at 4°C. Freshly prepared 100 mL of inoculum of *Micobacterium smegmatis* was spread in each dish and 20 mL (100 mg) solution of the test compound was poured in each well. Twenty milliliter DMSO was used as negative control. The plates were kept at 37°C for 24 h after which the diameter of the inhibition zones was measured [27]. Ciprofloxacin was used as a standard reference drug for comparison.



## 3. Results and discussion

It appears from the analytical data that the ligands react with metal salts in 1 : 2 (M : L) molar ratio to form complexes of general compositions $[M(INH\text{-}F)_2]Cl_2$ and $[M(IN\text{-}F)_2]$. The metal chloride interacts with the ligands in presence of a few drops of concentrated HCl to form $[M(INH\text{-}F)_2]Cl_2$. However, complexes of the type $[M(IN\text{-}F)_2]$ were obtained when reactions were carried out in the presence of aqueous $NH_4OH$. The reactions may be written as:

$$MCl_2 + 2INH\text{-}F \longrightarrow \left[M(INH\text{-}F)_2\right]Cl_2$$

$$MCl_2 + 2INH\text{-}F + 2NH_4OH \longrightarrow \left[M(IN\text{-}F)_2\right] + 2NH_4Cl + 2H_2O$$

where M = Pd(II) and Pt(II) and INH-F is the isoniazid Schiff base. The reactions proceed easily and all the complexes are soluble in DMSO, DMF, and $CHCl_3$. The molar conductance values of $10^{-3}$ mol L$^{-1}$ solutions of $[M(IN\text{-}F)_2]$ complexes lie in the range 12–15 Ohm$^{-1}$ cm$^2$ mol$^{-1}$ in dry DMF indicating nonelectrolytes. However, $[M(INH\text{-}F)_2]Cl_2$ complexes are 1 : 2 electrolytes, with conductance values of 205–220 Ohm$^{-1}$ cm$^2$ mol$^{-1}$. The metal complexes are diamagnetic, as expected for square planar $d^8$ complexes. Their magnetic susceptibilities lie in the range 0.3–0.8 $\times$ $10^{-6}$ c.g.s. units.

Table 2. Electronic spectral data ($cm^{-1}$) of the palladium(II) and platinum(II) complexes.

| Complex | Spectral bands ($cm^{-1}$) | Transitions | $\Delta_1$ ($cm^{-1}$) | $\Delta_2$ ($cm^{-1}$) | $\Delta_3$ ($cm^{-1}$) | $\nu_2/\nu_1$ |
|---|---|---|---|---|---|---|
| $[Pd(INH\text{-}F^1)_2]Cl_2$ | 22,222 | $^1A_{1g} \rightarrow {}^1A_{2g}$ ($\nu_1$) | 24,322 | 3368 | 1976 | 1.09 |
| | 24,390 | $^1A_{1g} \rightarrow {}^1B_{1g}$ ($\nu_2$) | | | | |
| | 26,666 | $^1A_{1g} \rightarrow {}^1E_{1g}$ ($\nu_3$) | | | | |
| $[Pd(IN\text{-}F^1)_2]$ | 21,598 | $^1A_{1g} \rightarrow {}^1A_{2g}$ ($\nu_1$) | 23,698 | 3530 | 2653 | 1.10 |
| | 23,923 | $^1A_{1g} \rightarrow {}^1B_{1g}$ ($\nu_2$) | | | | |
| | 26,881 | $^1A_{1g} \rightarrow {}^1E_{1g}$ ($\nu_3$) | | | | |
| $[Pt(INH\text{-}F^2)_2]Cl_2$ | 19,230 | $^1A_{1g} \rightarrow {}^1A_{2g}$ ($\nu_1$) | 21,330 | 5779 | 4876 | 1.23 |
| | 23,809 | $^1A_{1g} \rightarrow {}^1B_{1g}$ ($\nu_2$) | | | | |
| | 28,985 | $^1A_{1g} \rightarrow {}^1E_{1g}$ ($\nu_3$) | | | | |
| $[Pt(IN\text{-}F^2)_2]$ | 19,120 | $^1A_{1g} \rightarrow {}^1A_{2g}$ ($\nu_1$) | 21,220 | 5609 | 4906 | 1.23 |
| | 23,529 | $^1A_{1g} \rightarrow {}^1B_{1g}$ ($\nu_2$) | | | | |
| | 28,735 | $^1A_{1g} \rightarrow {}^1E_{1g}$ ($\nu_3$) | | | | |

The complexes are monomers as revealed by their molecular weight determinations. The data from analytical and physicochemical studies were correlated to explain the properties and natures of the complexes (table 1).



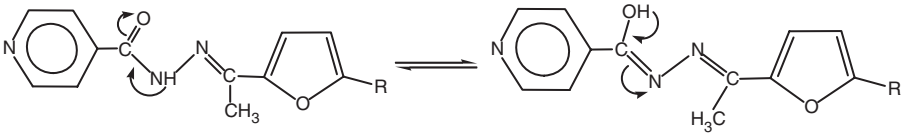
### 3.1. *Spectroscopic characterization*

**3.1.1. Electronic spectra.** The electronic spectra of the metal complexes were recorded in distilled DMSO so as to confirm their square-planar geometry. The spectra of the complexes show three bands due to three d–d spin-allowed transitions from the three lower lying "d" orbitals to the empty $d_{x^2-y^2}$ orbital. The ground state is $^1A_{1g}$ and excited states corresponding to the above transitions are $^1A_{2g}$, $^1B_{1g}$, and $^1E_{1g}$ in order of increasing energy. By assuming a value of $F_2 = 10F_4 = 600\,cm^{-1}$ for Slater Condon interelectronic repulsion parameters ($F_2$ and $F_4$), and subsequently the equations suggested by Gray and Ballhausen [28], it is possible to calculate the single electron parameters: The $\nu_2/\nu_1$ values (table 2) lie in the 1.09–1.23 range which are in close agreement with those reported earlier for square-planar complexes [28, 29].

**3.1.2. IR spectra.** A comparison of the IR spectra of the complexes and the ligands $INH\text{-}F^1$ and $INH\text{-}F^2$ shows that $\nu$(C=O) of the ligands at 1665–1675 $cm^{-1}$ and $\nu$(NH) at 3240–3246 $cm^{-1}$ are absent in the spectra of the respective complexes (table 3). This is presumably due to amide–imidol tautomerism (figure 2) and their subsequent coordination through the imidol oxygen [30]. This is supported by appearance of a new band in spectra of the complexes at 1520–1550 $cm^{-1}$ attributed to the azine group, >C=N–N=C<, which is absent in spectra of the hydrazones. Bands at 1600–1612 $cm^{-1}$ due to $\nu$(C=N) of the hydrazones shifts to lower wavenumbers indicating coordination of the azomethine nitrogen. Nonligand bands at 415–428 and 352–456 $cm^{-1}$ have been assigned to $\nu$(M–O) and $\nu$(M–N), respectively. In both hydrazones bands at 1480–1500 $cm^{-1}$ due to the pyridine ring nitrogen remain unchanged on complexation, indicating noninvolvement of the ring nitrogen in complex formation [31]. The overall IR spectral evidence suggests that both ligands are bidentate, coordinating through amide-oxygen and azomethine-nitrogen forming a five-membered chelate ring.

Table 3. IR ($cm^{-1}$) spectral data of the ligands and their metal complexes.

| | IR spectral data ($cm^{-1}$) | | | | |
|---|---|---|---|---|---|
| Compound | $\nu$(NH) | $\nu$(C=O) | $\nu$(C=N) | >C=N–N=C< | $\nu$(M → O) |
| INH-$F^1$ | 3240 | 1665 | 1600 | – | – |
| INH-$F^2$ | 3246 | 1675 | 1612 | – | – |
| $[Pd(INH\text{-}F^1)_2]Cl_2$ | – | 1290 | 1590 | 1520 | 415 |
| $[Pd(IN\text{-}F^1)_2]$ | – | 1293 | 1585 | 1526 | 418 |
| $[Pd(INH\text{-}F^2)_2]Cl_2$ | – | 1320 | 1595 | 1537 | 422 |
| $[Pd(IN\text{-}F^2)_2]$ | – | 1318 | 1588 | 1540 | 425 |
| $[Pt(INH\text{-}F^1)_2]Cl_2$ | – | 1298 | 1592 | 1542 | 423 |
| $[Pt(IN\text{-}F^1)_2]$ | – | 1300 | 1589 | 1546 | 424 |
| $[Pt(INH\text{-}F^2)_2]Cl_2$ | – | 1309 | 1597 | 1548 | 423 |
| $[Pt(IN\text{-}F^2)_2]$ | – | 1312 | 1594 | 1550 | 428 |

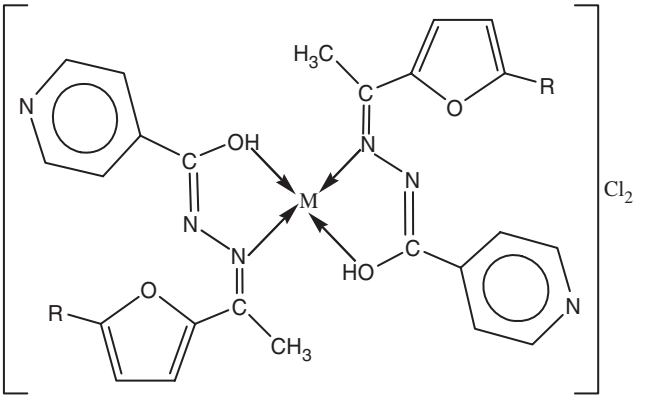



Amide form Imidol form

Figure 2. Tautomeric form of the ligands.

**3.1.3. $^1$H NMR spectra.** The $^1$H NMR spectral data of the ligands and their corresponding metal complexes were recorded in DMSO-$d_6$ with TMS as an internal standard. A comparison of $^1$H NMR spectra of the metal complexes and their respective ligands is shown in Supplementary material. Disappearance of the –NH proton signal at $\delta$ 10.06–11.02 ppm in the complexes is considered as an additional evidence of enolization of the ligand during complexation. A singlet at 8.90–8.92 ppm may be assigned to $\alpha$-protons of the pyridine ring. The multiplet at 6.97–8.27 ppm may be assigned to $\beta$-protons of the pyridine ring and protons of the aromatic ring. In the spectrum of the metal complexes, the signals due to $\alpha$- and $\beta$-protons of the pyridine ring and protons of the aromatic ring were downfield. A sharp singlet at $\delta$ 2.34–2.51 ppm due to the methyl protons attached to azomethine of the ligands undergoes a downfield shift due to the coordination of the azomethine nitrogen.

**3.1.4. $^{13}$C NMR spectra.** $^{13}$C NMR data recorded for both the ligands and their complexes support the proposed structures (figure S1 in Supplementary material and figure 3). Signals due to carbons attached to the azomethine nitrogen and enolic oxygen appear at $\delta$ 157.60–162.82 and $\delta$ 166.62–175.78 ppm, respectively. However, in the spectra of corresponding metal complexes, a considerable downfield shift is observed in these signals. The shifts in the positions of carbons adjacent to the coordinating atoms clearly indicate bonding of the azomethine nitrogen and enolic oxygen to the metal. Thus, the $^1$H and $^{13}$C NMR spectra confirm the monobasic bidentate nature of the ligands, already suggested by the IR spectral studies.




Addition complex

Figure 3. Proposed structures for addition complexes, M = Pd(II) and Pt(II); R = H(INH-$F^1$) and $CH_3$(INH-$F^2$).



**3.1.5. Mass spectra.** Mass spectroscopy, which is mainly applied in the analysis of biomolecules, has been increasingly used as a powerful structural characterization technique in coordination chemistry. The FAB mass spectrum of the $[Pd(INH\text{-}F^1)_2]Cl_2$ complex **3** was studied as a representative case. Peaks of appreciable intensity were observed at $m/z$ values 635.9, 564.5, and 229.3 (Supplementary material). The molecular ion peak for the complex $[Pd(INH\text{-}F^1)_2]Cl_2$ observed at $m/z$ 635.9 is in good agreement with its molecular weight, which suggests the monomeric nature of the complex and confirms the proposed formula. The peak at $m/z$ 564.5 indicates that the molecular ion gave a fragment ion $[Pd(INH\text{-}F^1)_2]^+$ by losing two chlorines. This fragment ion undergoes demetallation to form the species $[INH\text{-}F^1]^+$ that gave fragment ion peak at $m/z$ 229.3 as follows:

$$\underset{M/Z=635.9}{\left[Pd(INH\text{-}F^1)_2\right]Cl_2} \xrightarrow{-2Cl} \underset{M/Z=564.5}{\left[Pd\,(INH\text{-}F^1)_2\right]^+} \xrightarrow{\text{dematallation}} \underset{M/Z=229.3}{[INH\text{-}F^1]^+}$$

Therefore, these results complemented previous characterizations of the complexes as being square planar with bidentate Schiff-base ligands.

The structures in figures 3 and 4 are proposed for the complexes.

### 3.2. *X-ray diffraction studies*

The possible geometry of the finely powdered product, $[Pd(INH\text{-}F^1)_2]Cl_2$, has been deduced on the basis of X-ray powder diffraction pattern (Supplementary material). The observed interplanar spacing values ("*d*" in Å) have been measured from the diffractogram of the compound and the Miller indices $h$, $k$, and $l$ have been assigned to each $d$ value and $2\theta$ angles are reported in Supplementary material. The results show that the compound belongs to "orthorhombic" crystal system [32] having unit cell parameters as $a = 25.420$, $b = 17.320$, $c = 10.220$, maximum deviation of $2\theta = 0.045^\circ$ and $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$.

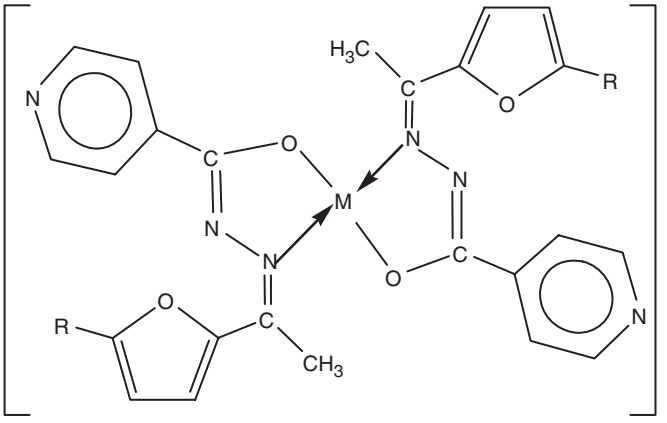



Figure 4. Proposed structures for substitution complexes, M = Pd(II) and Pt(II); R = H(INH-$F^1$) and $CH_3$(INH-$F^2$).



Table 4. Antifungal screening data for the ligands and their metal complexes.

| | (%) Inhibition after 96 h (Conc. in mg $mL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | *Aspergillus niger* | | | *Fusarium oxysporum* | | |
| Compound | 0.5 | 1.0 | 1.5 | 0.5 | 1.0 | 1.5 |
| INH-$F^1$ | 39 ± 0.4 | 54 ± 0.5 | 65 ± 0.5 | 40 ± 1.2 | 55 ± 0.6 | 67 ± 0.6 |
| INH-$F^2$ | 42 ± 0.3 | 57 ± 0.3 | 68 ± 0.6 | 42 ± 0.4 | 57 ± 0.3 | 69 ± 0.5 |
| [Pd(INH-$F^1$)$_2$]$Cl_2$ | 52 ± 0.1 | 62 ± 0.5 | 69 ± 0.4 | 50 ± 0.8 | 64 ± 0.5 | 70 ± 0.4 |
| [Pd(IN-$F^1$)$_2$] | 50 ± 0.6 | 60 ± 0.2 | 66 ± 0.5 | 48 ± 0.4 | 62 ± 0.8 | 69 ± 0.5 |
| [Pd(INH-$F^2$)$_2$]$Cl_2$ | 54 ± 0.3 | 65 ± 0.4 | 73 ± 0.5 | 52 ± 0.5 | 65 ± 0.6 | 74 ± 0.3 |
| [Pd(IN-$F^2$)$_2$] | 52 ± 0.3 | 63 ± 0.6 | 71 ± 0.3 | 50 ± 1.1 | 61 ± 0.5 | 72 ± 0.6 |
| [Pt(INH-$F^1$)$_2$]$Cl_2$ | 54 ± 0.4 | 65 ± 0.4 | 74 ± 0.5 | 55 ± 0.5 | 66 ± 0.2 | 78 ± 0.4 |
| [Pt(IN-$F^1$)$_2$] | 53 ± 0.5 | 62 ± 0.5 | 72 ± 0.5 | 53 ± 0.3 | 64 ± 0.5 | 76 ± 0.5 |
| [Pt(INH-$F^2$)$_2$]$Cl_2$ | 58 ± 0.7 | 69 ± 0.4 | 79 ± 0.3 | 59 ± 0.4 | 70 ± 0.3 | 80 ± 0.6 |
| [Pt(IN-$F^2$)$_2$] | 56 ± 1.1 | 67 ± 0.4 | 76 ± 0.7 | 56 ± 0.4 | 68 ± 0.4 | 77 ± 0.5 |
| Flucanazone | 82 ± 0.8 | 99 ± 0.4 | 100 ± 1.2 | 86 ± 0.5 | 98 ± 0.6 | 100 ± 0.6 |
| Control | – | – | – | – | – | – |

### 3.3. *Bioassay*

The antimicrobial studies showed that the ligands and their metal complexes exhibit considerable activity against all the pathogenic bacterial and fungal strains (tables 4 and 5). The results show that the complexes are more active than their parent ligands against the same microorganisms and as the concentration increases the activity also increases. MIC values (table 5) also show that metal complexes are more potent than both ligands. Chelation theory [33] accounts for the increased activity of the metal complexes. The enhanced activity could also be due to inherent properties of the metal ion in precipitating or denaturing proteins. As enzymes are proteins, it would be expected that the heavy metal would inactivate these catalysts [34].

The mode of action of antimicrobials may involve various targets in microorganisms, e.g. interference with cell wall synthesis, damage to the cytoplasmic membrane, causing

Table 5. Antibacterial screening data and MIC of the ligands and their metal complexes.

| | *Escherichia coli* (−) ($mg\,mL^{-1}$) | | | | | *Staphylococcus aureus* (+) ($mg\,mL^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Diameter of inhibition zone (in mm) | | % Activity Index | | | Diameter of inhibition zone (in mm) | | % Activity Index | |
| Compound | MIC ($mg\,mL^{-1}$) | 1.0 | 2.0 | 1.0 | 2.0 | MIC ($mg\,mL^{-1}$) | 1.0 | 2.0 | 1.0 | 2.0 |
| INH-$F^1$ | 0.7 | 6 ± 0.03 | 8 ± 0.08 | 46 | 50 | 0.6 | 8 ± 0.05 | 11 ± 0.09 | 57 | 61 |
| INH-$F^2$ | 0.6 | 7 ± 0.05 | 9 ± 0.06 | 53 | 56 | 0.5 | 9 ± 0.02 | 12 ± 0.02 | 64 | 66 |
| $[Pd(INH\text{-}F^1)_2]Cl_2$ | 0.4 | 10 ± 0.1 | 13 ± 0.1 | 76 | 81 | 0.4 | 11 ± 0.02 | 15 ± 0.04 | 78 | 83 |
| $[Pd(IN\text{-}F^1)_2]$ | 0.5 | 9 ± 0.1 | 12 ± 0.02 | 69 | 75 | 0.4 | 12 ± 0.08 | 16 ± 0.1 | 85 | 88 |
| $[Pd(INH\text{-}F^2)_2]Cl_2$ | 0.4 | 11 ± 0.05 | 14 ± 0.05 | 84 | 87 | 0.3 | 13 ± 0.02 | 17 ± 0.02 | 92 | 94 |
| $[Pd(IN\text{-}F^2)_2]$ | 0.5 | 10 ± 0.1 | 13 ± 0.1 | 76 | 81 | 0.4 | 12 ± 0.1 | 16 ± 0.1 | 85 | 88 |
| $[Pt(INH\text{-}F^1)_2]Cl_2$ | 0.3 | 11 ± 0.01 | 14 ± 0.08 | 84 | 87 | 0.3 | 12 ± 0.03 | 17 ± 0.08 | 85 | 94 |
| $[Pt(IN\text{-}F^1)_2]$ | 0.4 | 10 ± 0.06 | 12 ± 0.03 | 76 | 75 | 0.3 | 11 ± 0.06 | 15 ± 0.09 | 78 | 83 |
| $[Pt(INH\text{-}F^2)_2]Cl_2$ | 0.5 | 12 ± 0.08 | 15 ± 0.04 | 92 | 93 | 0.4 | 13 ± 0.02 | 17 ± 0.1 | 92 | 94 |
| $[Pt(IN\text{-}F^2)_2]$ | 0.4 | 10 ± 0.03 | 13 ± 0.01 | 76 | 81 | 0.3 | 11 ± 0.1 | 15 ± 0.08 | 78 | 83 |
| Ampicillin | 0.3 | 13 ± 0.08 | 16 ± 0.1 | 100 | 100 | 0.2 | 14 ± 0.1 | 18 ± 0.03 | 100 | 100 |
| Control | – | – | – | – | – | – | – | – | – | – |

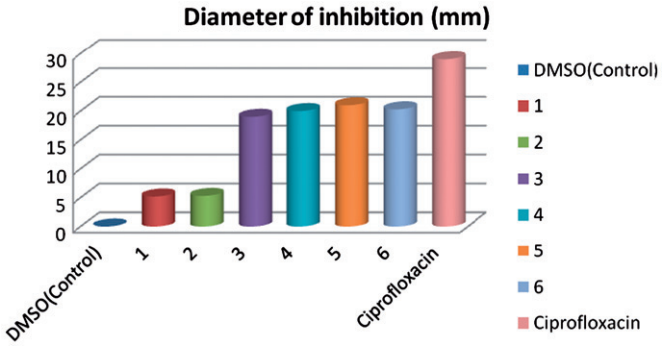



Figure 5. Antimycobacterial assay of the ligands and their metal complexes against *M. smegmatis*.

an alternation of the cell permeability, or a disorganization of the lipoproteins leading to cell death. Antimicrobials can bind to ribosome and may interfere with peptide chain formation in microorganisms or with the transcription mechanisms. At lower concentration, inhibition is less severe because the activities of the organisms will be slowed down, while at higher concentration, more enzymes will become inhibited leading to a quicker death of the organisms [35].

From bactericidal activity, it is apparent that the complexes were more active towards Gram (+) than Gram (−) strain. The reason is the difference in the structures of the cell walls. The walls of Gram (−) cells are more complex than those of Gram (+) cells. Lipopolysaccharides form an outer lipid membrane and contribute to the complex antigenic specificity of Gram (−) cells [36].

The antimycobacterial assay for the ligands and their metal complexes against *M. smegmatis* are shown in figure 5, which indicates that metal conjugation enhances the antitubercular activity of the parent ligand by three- or four-fold, making it comparable with that of Ciprofloxacin. Such an enhancement may be due to greater lipophilicity of the metal conjugates, promoting facile intracellular metal transport.

## 4. Conclusion

Based on various physicochemical and structural investigations, the ligands are bidentate forming square-planar complexes with Pd(II) and Pt(II). All the compounds showed appreciable antifungal and antibacterial activity. The present study shows that metal conjugation may be advantageous in designing highly effective drugs in antitubercular therapy.

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