Synthesis, Characterization, and Study of Anti-Tubercular and Anti-Microbial Activity of Isonicotinohydrazide Tridentate Schiff Base Ligands

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ABSTRACT: *New Schiff bases derived by the condensation of isonicotinoylhydrazide and 2-hydroxy-3-methoxy-benzaldehyde and 2,4-dihydroxybenzaldehyde have been synthesized. Compounds were characterized on the basis of various spectroscopic techniques like IR, ^{1}H and ^{13}C NMR studies, elemental analysis. The ligands were subjected to anti-microbial and anti-tubercular activity screening using serial broth dilution method and Minimum Inhibitory Concentration (MIC) is determined. The ligands were evaluated for their anti-microbial activity against Gram-positive bacteria (Staphylococcus aureus ATCC 9144) and Gram-negative bacterium (Escherichia coli ATCC 11303). Therefore, newly synthesized ligands showed good biological activity against tested bacteria. Compounds show high levels of activity against Mycobacterium tuberculosis (M. tuberculosis H37RV) in vitro.*

KEYWORDS: *Schiff base; Isonicotinohydrazide; Anti-tubercular; Anti-microbial.*

INTRODUCTION

Tuberculosis (TB), as a scourge of humanity for thousands of years, introduced a worldwide pandemic disease which mainly affected by *Mycobacterium tuberculosis* (MTB) [1]. Isoniazid, also known as isonicotinylhydrazide (INH), and its derivatives including *N*-containing heterocycles have gained prominence in medicinal chemistry due to their variety of biological activity such as anti-mycobacterial, anti-bacterial and virus, anti-fungal/tumor/analgesic/convulsant activities, as well [2-9].

Compounds containing azomethine group (–CH=N–) introduced as Schiff bases. Synthesis method as well as physical/chemical characteristics reported by many different researchers. Schiff-base ligands well known as a significant class of organic compounds due to their structural properties and pharmaceutical activities including antibacterial, anticancer, anti-tumor and also anti-inflammatory [10-15]. Hydrazones have drawn wide attention and been extensively applied in the field

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of chemistry due to their wide spectrum of pharmacological activity profile with structural flexibility as well as ligating behavior. Hydrazones which distinguished in the presence of two interlinked nitrogen atoms as R1CH=N–NH–COR2 are more interesting, because they can functionalize as antimicrobial, antitubercular and antitumor agents [16–19]. Some research work focused on various types of hydrazones in the field of medicinal, due to their wide biological activities, where INH hydrazide-hydrazone derivatives represented great anti-TB properties [2]. Many efforts have been done to develop new anti-TB agents, but there are not any reports about new drug for more than 5 decades. It also has been suggested that the incorporation of lipophilic moieties into the framework of INH can increase permeation of the drug among bacterial cells, thereby an enhancement of the anti-TB [20]. Hence, an attempt is necessary to improve the INH molecule via chemical modifications on core structure. This procedure can enhance biological responses against MTB., and reduce hepatotoxicity, which cause to circumvent resistance phenomena for both drug-resistant MTB., and nontuberculous mycobacteria (NTM). So, the aim of present study was focused on design, synthesis and identification of new Schiff base of isoniazid as new compounds which are able to attain the same clinical efficacy.

In this poin of view more attempts is necessary to achieve new compounds with better properties in the terms of antitubercular and antimicrobial activity (Fig. 1).

EXPERIMENTAL SECTION

Materials and Physical measurements

All the reagents used in the synthesis of ligand viz. 2-hydroxy-3-methoxybenzadehyde, 2,4-dihydroxybenzadehyde, isonicotinoylhydrazide(isoniazid) were purchased from Merck chemical and used without further purification. NMR spectra were recorded at ambient temperature with a BRUKER AVANCE 500 MHz spectrometer using DMSO as solvent, chemical shift values (δ) are given in ppm. Infrared spectra (4000–400 cm^{-1}) were recorded as KBr discs with an IR Prestige-21 Shimadzu FT-IR spectrophotometer. Microanalyses (C, H, N) of the ligands were carried out on a Leco CHNS elemental analyzer.

Synthesis of N′-(2-hydroxy-3-methoxybenzilidine) isonicotinohydrazide ligand (1)

3-methoxy-2-hydroxybenzilidine (1.52 g, 0.01 mol) was added to a solution of isonicotinohydrazide (1.37 g, 0.01 mol) in methanol (30 ml) and stirred for 3 h. The pale yellowish solid separated was filtered, washed repeatedly with methanol, dried in air and recrystallized from ethanol. Yield: 91%. ^{1}H NMR (500 MHz, [D_6] DMSO, 25 °C): δ = 12.07 (s, 1 H, -OH), 10.68 (s, 1 H, -NH), 8.67 (s, 1 H, -CHN), 8.75 [d, ^3J(H,H) = 5.55 Hz, 2 H, H (11,12)], 7.82 [d, ^3J(H,H) = 5.95 Hz, 2 H, H (10,13)], 7.14 [dd, ^3J(H,H) = 7.75 Hz, ^4J(H,H) = 0.95 Hz, 1 H, H (5)], 6.97 [dd, ^3J(H,H) = 7.90 Hz, ^4J(H,H) = 0.85 Hz, 1 H, H (3)], 6.79 [d, ^3J(H,H) = 7.90 Hz, 1 H, H (4)], 3.81 [s, 3 H, -OCH_3] ppm. ^{13}C NMR (500 MHz) δ = 161.1, 150.1, 149.0, 147.4, 147.0, 139.8, 121.3, 120.6, 119.0, 118.8, 115.3, 56.1 ppm. IR (KBr, cm^{-1}); 3205 ($\nu_{N–H}$); 1696 ($\nu_{C=O}$); 1607 ($\nu_{C=N}$); 1567, 1469, ($\nu_{C=C}$); 1259 (ν_{C-O}); 1044 (ν_{N-N}). Anal. Calc. for $C_{14}H_{13}N_3O_3$ (271.1) (%): C, 61.99; H, 4.83; N, 15.49. Found (%): C, 61.87; H, 4.78; N, 15.38.

Synthesis of (2,4-dihydroxybenzilidine) isonicotinohydrazide ligand (2)

2,4-dihydroxybenzaldehyde (1.38 g, 0.01 mol) was added to a solution of isonicotinohydrazide (1.37 g, 0.01 mol) in ethanol (30 ml) and stirred for 3 h. The orange solid separated was filtered, washed repeatedly with ethanol, dried in air and recrystallized from ethanol. Yield: 87%. ^{1}H NMR (500 MHz, [D_6] DMSO, 25 °C): δ = 12.10 (s, 1 H, -OH), δ = 11.25 (s, 1 H, -OH), 10.00 (s, 1 H, -NH), 8.52 (s, 1 H, -CHN), 8.76 [d, ^3J(H,H) = 4.70 Hz, 2 H, H (11,12)], 7.81 [d, ^3J(H,H) = 4.70 Hz, 2 H, H (10,13)], 7.35 [d, ^3J(H,H) = 8.45 Hz, 1 H, H (5)], 6.36 [dd, ^3J(H,H) = 8.45 Hz, ^4J(H,H) = 2.25 Hz, 1 H, H (4)], 6.32 [d, ^4J(H,H) = 2.25 Hz, 1 H, H (2)] ppm. ^{13}C NMR (125 MHz) δ = 161.0, 160.9, 159.5, 150.3, 150.0, 140.1, 131.2, 121.4, 110.4, 107.8, 102.6ppm. IR (KBr, cm^{-1}); 3292 ($\nu_{N–H}$); 1658 ($\nu_{C=O}$); 1605 ($\nu_{C=N}$); 1546, 1494, ($\nu_{C=C}$); 1271 (ν_{C-O}); 965 (ν_{N-N}). Anal calcd. for $C_{13}H_{11}N_3O_3$ (257.2) (%): C, 60.70; H, 4.31; N, 16.33. Found (%): C, 60.46; H, 4.19; N, 16.44.

Anti-microbial activity

Serial dilutions of test compounds were made in broth, after which a standardized microorganism suspension was added. Quantities of test compounds were serially diluted

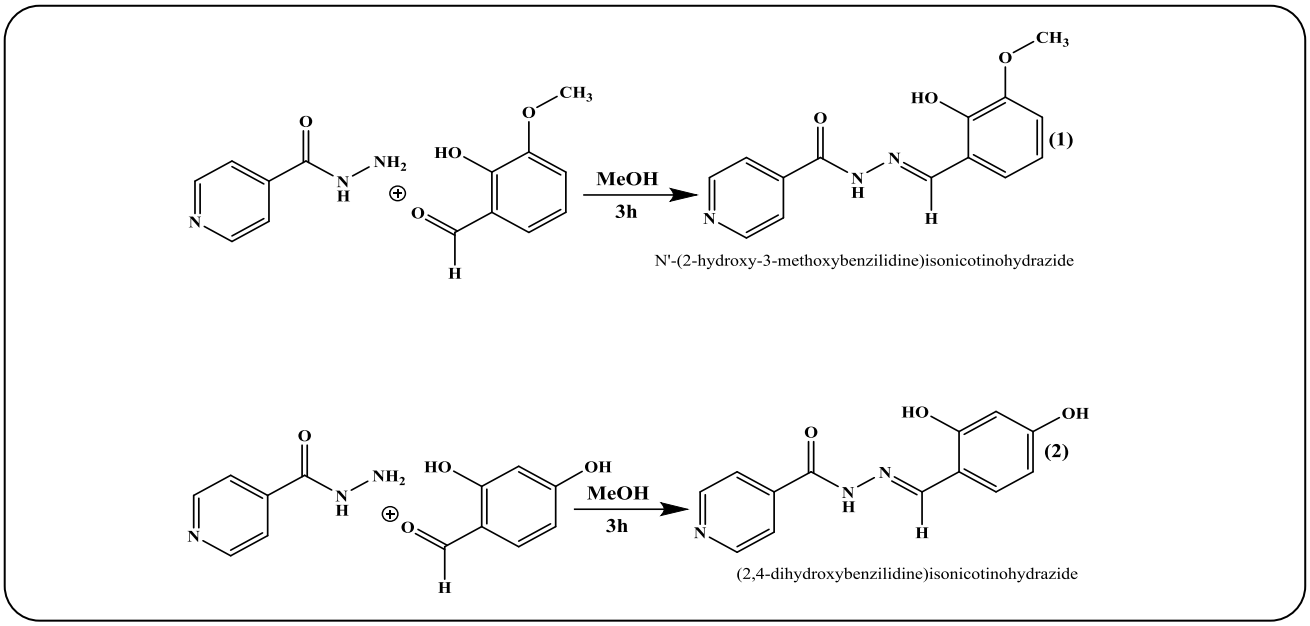

Fig. 1: Synthetic route for the preparation of Schiff base ligands.

to attain the final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/ml. Each of the 10 test tubes was inoculated with suspension of microorganism (1×106 CFU/ml) to be tested and incubated at 37◦C for 24 h. Cloudiness in the test tubes indicated that microorganism growth have not inhibited by the antibiotic contained in the medium at the test concentration.

Anti-tubercular activity

Test compounds were evaluated for in vitro anti-mycobacterium activity. The MIC was determined and interpreted for M. tuberculosis H37Rv according to the procedure of the approved microdilution reference method of antimicrobial susceptibility testing [21]. Compounds were taken at concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/ml in 2% DMF. M. tuberculosis H37Rv strain was used in Middle brook 7H-9 broth which was inoculated with standard as well as test compound and incubated at 37◦C for 4 weeks. The bottles were inspected for growth twice a week for a period of 3 weeks. Reading was taken at the end of fourth week. The appearance with turbidity of 1×106 CFU/ml was considered as bacterial growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle. Test compound was compared to reference drugs Isoniazid (MIC=0.025 μg/ml). The antimicrobial and anti-tubercular activity test was run in triplicate.

RESULTS AND DISCUSSION

The elemental analyses of the ligands are in consistence with the molecular formula $C_{14}H_{13}N_3O_3$ (1) and $C_{13}H_{11}N_3O_3$ (2). Ligands are soluble in solvents like DMF and DMSO but insoluble in common organic solvents. Anal. Calc. for $C_{14}H_{13}N_3O_3$ (**1**) (271.1) (%):C, 61.99; H, 4.83; N, 15.49. Found (%): C, 61.87; H, 4.78; N, 15.38 and Anal. Calc. for $C_{13}H_{11}N_3O_3$ (**2**) (257.2) (%):C, 60.70; H, 4.31; N, 16.33. Found (%): C, 60.46; H, 4.19; N, 16.44.

Infrared Spectroscopy

The IR spectrum of the ligand (1) exhibit two bands in the regions 3205 and 1696 cm^{-1} due to ν(NH) and ν(C=O) stretches. A new band appearing in the 1259 cm^{-1} is assigned to the ν(C-O) (enolic) mode. The band due to the imine group ν(C=N) mode of ligand at 1607 cm^{-1} and IR spectrum for ligand (2) exhibit two bands in the regions 3292 and 1658 cm^{-1} due to ν(NH) and ν(C=O) stretches. A new band appearing in the 1271 cm^{-1} is assigned to the ν(C-O) (enolic) mode. The band due to the imine group ν(C=N) mode of ligand at 1605 cm^{-1}. The IR band assignments of ligands are included in Table 1.

NMR Spectroscopy

Typical proton NMR spectra of the ligands were recorded at room temperature using DMSO as the solvent and the data were summarized in ‘‘Experimental’’ section.

Table 1: Important IR bands (cm^{-1}) of compounds.

ligand	(1)	(2)
ν(NH)	3205	3292
ν(C=O)	1696	1658
ν(C=N)	1607	1605
ν(C-O)	1259	1271
ν(N-N)	1044	965

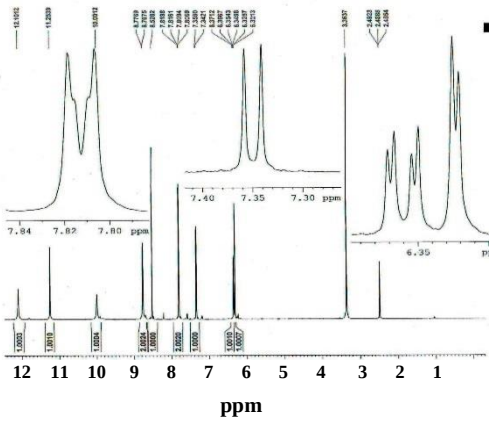

Fig. 2: ^{1}H NMR spectrum of 2 in DMSO.

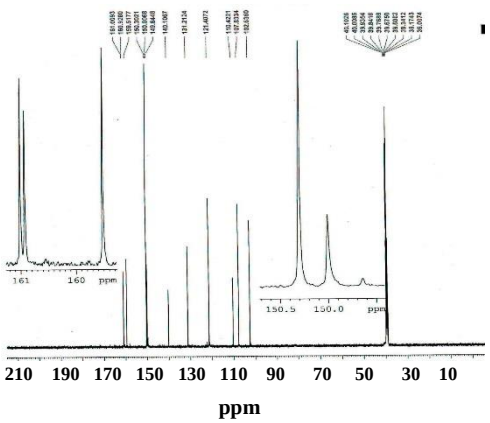

Fig. 3: ^{13}C NMR spectrum of 2 in DMSO.

In the ^{1}H NMR spectrum of ligand (1) a signal at δ 12.07 ppm is assignable to the -OH proton, while a signal at δ 10.68 ppm is assigned to the -NH proton. Furthermore, azomethine proton is observed as a singlet at δ 8.67 ppm. Aromatic protons of the ligand appear well within the expected range. The aromatic protons of the ligand appeared at 6.79–8.75 ppm. Aromatic protons of the ligand appear well within the expected range. A silglet at δ 3.81 ppm is assigned to the–OCH_3 protons. In the ^{1}H NMR spectrum of ligand (2) a signal at δ 12.10 ppm and 11.25 ppm are assignable to the -OH protons, while a signal at δ 10.00 ppm is assigned to the -NH proton. Furthermore, azomethine proton is observed as a singlet at δ 8.52 ppm. Aromatic protons of the ligand appear well within the expected range. The aromatic protons of the ligand appeared at 6.32–8.77 ppm. Aromatic protons of the ligand appear well within the expected range (Fig. 2). Also, ^{13}C NMR showed 11 carbons for this structure. The ^{13}C NMR spectrum of the ligand (2) shows the two carbonyl and iminic carbon as functional group signals at 161.0 and 140.1 ppm, respectively (Fig. 3).

Anti-microbial activity

The synthesized ligands were screened for the inhibition of microbial growth under standard conditions, which utilized for demonstrating their antimicrobial efficacy using a serial broth micro dilution method in 96 multi-cell micro liter plates [22]. Ligands are tested at different concentrations and Minimum Inhibitory Concentration is determined.

Anti-bacterial activity

The antibacterial activities of synthesized compounds are tested against bacteria like *Staphylococcus aureus* (SA, ATCC 9144), *Escherichia coli* (EC, ATCC 87261). The results are compared with standard antibacterial drug Isoniazid (Table 2).

Ceftriaxone is used in the treatment of infections caused by drug-sensitive and gram-negative bacteria, skin infections, and urinary tract infections. Ceftazidime is used to treat infections caused by gram-positive and gram-negative drug-sensitive bacteria, including bile duct infections, respiratory infections, and infectious disease treatment in patients whose immune systems are weakened.

Table 2: In vitro antimicrobial activity of the compounds and standard drug (MIC in µg/mL).

Compound	Gram positive bacteria (S. aurous ATCC 9144)	Gram negative bacteria (E. coli ATCC 11303)	Mycobacterium Tuberculosis (M. tuberculosis $H_{37}RV$)
Ligand (1)	8	4	4
Ligand (2)	16	8	8
Ceftriaxone	0.010	0.015	-
Ceftazidime	0.015	0.020	-
Isoniazid	-	-	0.025

Anti-tubercular activity

As the ligands are a derivative of Isoniazid, were tested for anti-tubercular activity in order to compare with the activity of isoniazid (Table 2). The anti-mycobacterium activities of the synthesized compounds are assessed against M. tuberculosis H37RV at 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL. The Minimum Inhibitory Concentrations of compounds compared with Isoniazid, the standard anti-TB drugs and are summarized in Table 2. Ligands show inhibition at concentration 4 µg/mL for (1) and 8 µg/mL for (2).

CONCLUSIONS

In this work, new tridentate hydrazone Schiff base ligands were synthesized by condensation of 3-methoxysalicyaldehyde and 2,4-dihydroxybenzaldehyde with isonicotinhydrazide in refluxing methanol and characterized on the basis of physico-chemical and spectral (^{1}H NMR, ^{13}C NMR, IR) studies. The compounds were readily prepared for evaluation against M. tuberculosis in good yield. The antimicrobial activity results showed that the synthesized Schiff base compounds possess a good antibacterial activity against both gram-negative and gram-positive bacteria tested. Both Schiff base ligands (1 and 2) showed more antibacterial effect on gram-negative bacteria than gram-positive bacteria.

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