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An efficient synthesis, anticancer and antimycobacterial activities of new substituted pyridine based azomethine derivatives

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RESEARCH ARTICLE

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ABSTRACT

Pyridine, a fundamental heterocyclic scaffold, is a key structural component in numerous biologically active molecules, including alkaloids, vitamins B3 and B6, coenzymes, and other natural products. Its significance in medicinal chemistry arises from its versatile physicochemical properties, such as its capacity to form hydrogen bonds, its high water solubility, and its chemical stability. In this study, a series of substituted pyridine-based analogues (3a-h) were synthesized and their structural elucidation was performed using various spectroscopic techniques. These derivatives incorporate an azomethine functionality within the pyridine core. The structural characterization of the newly synthesized compounds was achieved through spectroscopic analyses, including mass spectrometry, ^{1}H NMR, ^{13}C NMR, infrared (IR) spectroscopy, and complementary analytical methods such as solubility and melting point determination. The biological evaluation of the derivatives 3a-h was carried out to assess their in vitro cytotoxic activity against the human colon cancer cell line HCT-15 and the breast cancer cell line MCF-7 using the sulforhodamine B (SRB) assay. The results indicated that the synthesized compounds exhibited an anticancer activity ranging from moderate to promising. Furthermore, the compounds were subjected to preliminary antituberculosis (anti-TB) screening against *Mycobacterium bovis*, a representative strain of *Mycobacterium tuberculosis*, at varying concentrations.

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1. Introduction

Cyclic organic compounds that have at least one heteroatom are known as heterocyclic compounds [1]. The most common heteroatoms in these compounds are nitrogen, oxygen, and sulfur, but heterocyclic rings with additional heteroatoms are also well known [2,3]. A carbocyclic compound is an organic compound that is cyclic and has all its carbon atoms arranged in rings [4-6]. Due to their ability to treat a variety of diseases, heterocyclic compounds are considered one of the essential classes of organic compounds and are utilized in many biological fields [7]. Over the past several decades, numerous chemically synthesized compounds have been used to develop anticancer drugs. More than 75% of materials used in clinical settings today are heterocyclic derivatives, which are compounds made up of at least two distinct elements [8-11]. These compounds have attracted a great deal of attention in the development of pharmacologically active molecules and advanced organic materials [12,13]. Multidrug-resistant bacterial infections are becoming more common at an alarming rate, and doctors are now increasingly relying on Vancomycin® as an antibiotic for serious infections resistant to conventional agents. This suggests that new classes of antimicrobial agents must be developed [14-16]. Researchers and medicinal chemists are always interested in heterocyclic compounds containing sulfur, nitrogen and / or oxygen such as pyrazole and thiophene due to their numerous biological and pharmacological uses [17].

Pyridine is a member of a very important class of heterocyclic compounds that have a significant impact on medicine [18]. Due to its numerous incorporations of medicinal compounds, it has become a versatile heterocyclic compound with a wide range of biological activities (Figure 1). Trigonelline, alkaloids, coenzymes, natural antibiotics such as pyridomycin, vitamin B3 and B6 contain the pyridine nucleus, a key heterocyclic scaffold [19]. Furthermore, the aqueous solubility, metabolic stability, and lipophilicity of the target drug can all be changed by pyridine nuclei. As a result, the creation of novel derivatives based on pyridines has garnered significant interest in the treatment of various diseases [20].

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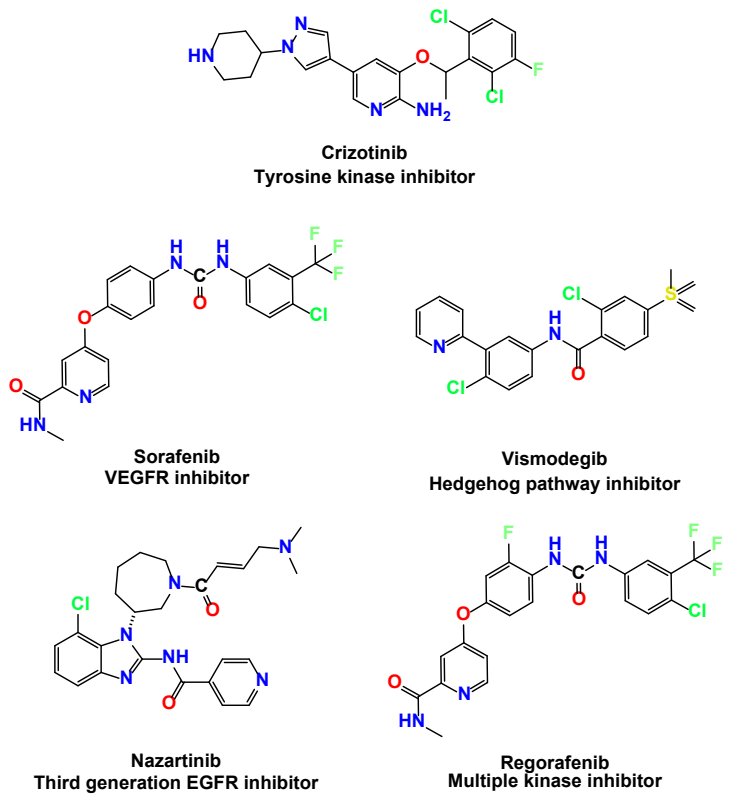


Figure 1. Pyridine-based drug molecules.

In this study, we report the synthesis of a series of novel pyridine-based Schiff bases derived from substituted pyridine cores, considering the pivotal role of pyridine as a biologically active scaffold with broad-spectrum pharmacological potential. Synthesized compounds were evaluated for their anti-tuberculosis (anti-TB) and anticancer activities using *in vitro* assays. Structural characterization was performed using electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) and Fourier-transform infrared (FT-IR) spectroscopy.

2. Experimental

2.1. Materials and instrumentations

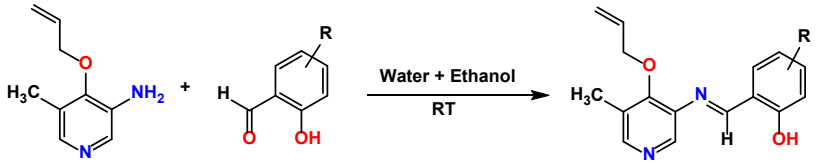
Merck, Aldrich, and S.D. Fine Chemical Limited, Mumbai, provided all the chemicals and solvents. Before use, all solvents were dried and distilled. Thin-layer chromatography, utilizing pre-coated aluminum sheets with GF_{254} silica gel and a 0.2 mm layer thickness (E. Merck) and solvent systems of toluene: methyl acetate: methanol (5:4:1, v:v:v) as used to monitor the reactions. The melting points of the synthesized compounds were determined using a Veego VMP-MP melting point apparatus. Infrared spectra were recorded on a Shimadzu FTIR-8400S spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were acquired at the Sophisticated Analytical Instrumentation Facility (SAIF), Punjab University, Chandigarh, using a Bruker Avance-II 400 NMR spectrometer operating at 400 MHz, with tetramethylsilane (TMS) as an internal reference in dimethylsulfoxide (DMSO-d_6). Chemical shifts are reported in δ units (ppm). Ultraviolet (UV) spectral analysis was performed using a Shimadzu 1700 UV-vis spectrophotometer. Time-of-flight mass spectrometry with electrospray ionization (LC-MS Spectrometer Model Q-ToF Micro Waters) spectra were recorded at 70 eV.

2.2. Synthesis

General procedure for the synthesis of pyridine-based azomethine derivatives 3a-h: In the round bottom flask substituted 3-aminopyridine (0.0304 mol) and 4 mL of water were mixed together and in the resulting suspension substituted ortho hydroxybenzaldehydes (a: 2-hydroxybenzaldehyde, b: 2-hydroxy-4-nitrobenzaldehyde, c: 4-formyl-3-hydroxybenzamide, d: 2-hydroxy-5-methoxy-4-methylbenzaldehyde, e: 4-chloro-2-hydroxybenzaldehyde, f: 2-hydroxy-4-methylbenzaldehyde, g: 2-hydroxy-3-nitrobenzaldehyde, h: 2-chloro-6-hydroxybenzaldehyde) in ethyl alcohol (0.0306 mol) with stirring. The reaction was carried out over 2 hours at 30-32 °C. After completing the reaction, the mixture was stirred for 20 min excess and the solid product formed was filtered off and washed with water. The crude azomethine compound was recrystallized with ethanol to obtain a pure compound (Figure 2).

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl) phenol (**3a**): Color: Brown. Yield: 83%. M.p.: 140-142 °C. FT-IR (KBr, ν, cm^{-1}): 3265-3675 (O-H, broad band), 1572-1644 (C=N), 1450-1490 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.38-3.37 (d, 2H, -CH_2-), 3.67-3.63 (s, 3H, -CH_3), 5.16-5.06 (d, 2H, -CH_2), 6.04-5.96 (s, 1H, OH), 6.97-6.94 (t, 1H, C=CH), 7.23-7.21 (s, 1H, ArH), 7.60-7.53 (s, 3H, ArH), 8.02-8.00 (s, 2H, Py-H), 10.92-10.90 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.52, 56.55, 77.05, 110.20, 116.52, 116.83, 129.25, 130.21, 131.04, 137.45, 130.00, 143.45, 149.00, 161.33. MS (ESI, *m/z*) calculated for $C_{16}H_{16}N_2O_2$:268.31; found: 269.12.

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-5-nitrophenol (**3b**): Color: Yellow. Yield: 88%. M.p.: 136-138 °C. FT-IR (KBr, ν, cm^{-1}): 3280-3682 (O-H, broad band), 1582-1655 (C=N), 1435-1482 (C=C), 1320 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.43-3.41 (d, 2H, CH_2), 3.89-3.90 (s, 3H, CH_3), 5.10-5.14 (d, 2H, CH_2), 6.00-5.90 (s, 1H, OH), 6.85-6.83 (t, 1H, C=CH), 7.37-7.26 (s, 1H, Ar H), 7.95-7.90 (s, 2H, Ar H), 8.35-8.34



1 2 3a-h

Compound	R	Compound	R
3a	H	3e	5-Cl
3b	5-NO_2	3f	5-CH_3
3c	3-CO-NH_2	3g	6-NO_2
3d	5-CH_3 and 4 *o*-CH_3	3h	3-Cl

Figure 2. Synthesis of pyridine-based azomethine derivatives 3a-h.

(s, 2H, Py-H), 12.10-12.09 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.51, 36.49, 76.00, 77.05, 77.39, 116.50, 116.93, 122.47, 123.97, 125.00, 131.46, 136.72, 137.44, 143.45, 149.41, 149.97, 153.57. MS (ESI, *m/z*) Calculated for $C_{16}H_{15}N_3O_4$: 313.31; found: 314.11.

4-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-3-hydroxybenzamide (**3c**): Color: White. Yield: 92%. M.p.: 138-140 °C. FT-IR (KBr, ν, cm^{-1}): 3210-3590 (O-H, broad band), 1580-1645 (C=N), 1420-1490 (C=C), 1688 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.43-3.32 (s, 2H, CH_2), 3.91-3.83 (s, 3H, CH_3), 5.16-5.08 (d, 2H, CH_2), 6.04-5.96 (s, 1H, OH), 7.19-7.00 (s, 2H, NH_2), 7.19-7.16 (t, 1H, C=CH), 8.11-8.06 (s, 3H, Ar H), 8.19-8.15 (s, 2H, Py H), 10.73-10.71 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.51, 55.92, 111.90, 115.03, 115.96, 122.37, 129.40, 130.32, 135.92, 137.92, 137.43, 139.43, 144.27, 144.64, 153.00, 167.12. MS (ESI, *m/z*) Calculated for $C_{17}H_{17}N_3O_3$: 311.34; found: 312.13.

2-((E)-(4-(allyloxy)-5-methylpyridin-3-ylimino) methyl)-4-methoxy-5-methylphenol (**3d**): Color: Creamy. Yield: 81%. M.p.: 138-140 °C. FT-IR (KBr, ν, cm^{-1}): 3220-3570 (O-H, broad band), 1530-1610 (C=N), 1450-1498 (C=C), 1090 (O-CH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.30-2.26 (s, 3H, CH_3), 3.39-3.34 (s, 3H, CH_3), 3.90-3.82 (s, 3H, OCH_3), 3.94-3.90 (d, 2H, =CH_2), 5.17-5.06 (s, 2H, CH), 6.04-5.99 (s, 1H, OH), 6.95-6.94 (t, 1H, =CH) δ 7.19-7.17 (s, 2H, ArH), 7.59-7.26 (s, 2H, Py), 12.06-12.07 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.95, 39.40, 55.94, 56.08, 113.00, 115.50, 120.02, 129.07, 130.16, 133.76, 137.42, 137.45, 137.99. 142.03, 149.61, 153.91. MS (ESI, *m/z*) Calculated for $C_{18}H_{20}N_2O_3$: 312.14; found: 313.15.

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-5-chlorophenol (**3e**): Color: Brown. Yield: 89%. M.p.: 144-146 °C. FT-IR (KBr, ν, cm^{-1}): 3200-3650 (O-H, broad band), 1570-1650 (C=N), 1430-1480 (C=C), 680 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.52-2.51 (s, 3H, CH_3), 3.39-3.90 (d, 2H, CH_2), 5.14-5.06 (d, 2H, CH_2), 6.01-5.96 (s, 1H, OH), 6.89-6.90 (t, 1H, C=CH), 7.15-7.14 (s, 1H, ArH), 7.63-7.61 (s, 2H, ArH), 8.04-8.01 (s, 2H, PyH), 10.55-10.54 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.44, 54.88, 55.00, 111.03, 116.64, 125.16, 128.77, 129.20, 135.55, 136.66, 137.15, 139.90. 143.53, 150.15, 154.00. MS (ESI, *m/z*) Calculated for $C_{16}H_{15}ClN_2O_2$: 302.76; found: 303.09.

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-5-methylphenol (**3f**): Color: Creamy. Yield: 87%. M.p.: 142-144 °C. FT-IR (KBr, ν, cm^{-1}): 3264- 3670 (O-H, broad band), 1560-1644 (C=N), 1400-1470 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.61-2.51 (s, 3H, CH_3), 3.30-3.04 (d, 2H, CH_2), 3.95-3.74 (s, 3H, CH_3), 5.16-5.10 (d, 2H, CH_2), 6.01-5.97 (s, 1H, OH), 6.97-6.96 (t, 1H, C=CH), 7.23-7.22 (s, 1H, Ar H), 7.35-7.32 (s, 1H, Ar H), 7.79-7.70 (s, 1H, ArH), 7.42-7.34 (s, 2H, PyH), 11.38-11.37 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.56, 55.90, 115.40, 115.53, 115.60, 115.90, 128.69, 130.30, 131.11, 131.31, 136.51, 137.47, 138.23, 142.95, 148.88, 149.88. MS (ESI, *m/z*) Calculated for $C_{17}H_{18}N_2O_2$: 282.137; found: 283.149.

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-6-nitrophenol (**3g**): Color: Yellow. Yield: 86%. M.p.: 136-138 °C. FT-IR (KBr, ν, cm^{-1}): 3260-3672 (O-H, broad band), 1575-1641 (C=N), 1424-1477 (C=C), 1084 (O-CH_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.51-2.53 (s, 3H, CH_3), 3.37-3.77 (d, 2H, CH_2), 5.10-5.17 (d, 2H, CH_2), 6.11-6.88 (s, 1H, OH), 6.92-6.98 (t, 1H, C=CH), 7.18-7.20 (s, 1H, Ar H), 7.65-7.68 (s, 2H, ArH), 8.04-8.09 (s, 2H, PyH), 10.58-10.56 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.55, 54.89, 55.14 111.13, 116.68, 125.20, 1287.78, 129.45, 135.65, 136.70, 137.19, 139.80. 143.51, 150.19, 154.20. MS (ESI, m/z) Calculated for $C_{16}H_{15}N_3O_4$: 313.106; found 314.11 [M+].

2-((E)-(4-(Allyloxy)-5-methylpyridin-3-ylimino) methyl)-3-chlorophenol (**3h**): Color: Yellow. Yield: 81%. M.p.: 140-142 °C. FT-IR (KBr, ν, cm^{-1}): 3260-3672 (O-H, broad band), 1575 -1641 (C=N), 1424-1477 (C=C), 1084 (O-CH_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.57-2.52 (s, 3H, CH_3), 3.37-3.35 (d, 2H, CH_2), 5.11-5.09 (d, 2H, CH_2), 6.11-5.99 (s, 1H, OH), 6.99-6.97 (t, 1H, C=CH), 7.16-7.13 (s, 3H, ArH), 7.61-7.60 (s, 2H, Py H), 10.50-10.49 (s, 1H, N=CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.10, 54.16, 55.88, 111.30, 116.89, 125.80, 128.67, 129.39, 135.59, 136.55, 137.27, 139.89. 143.03, 150.55, 154.35. MS (ESI, *m/z*) Calculated for $C_{16}H_{15}ClN_2O_2$: 302.082; found: 303.09.

2.3. In vitro cancer activity

All newly synthesized compounds were evaluated for their *in vitro* anticancer and antimycobacterial activity. *In vitro* tests for drugs anticancer activity evaluation were performed at the Anticancer Drug Screening Facility (ACDSF) at the Tata Memorial Center for Cancer Treatment, Research and Education (ACTREC), Navi Mumbai, India.

In vitro anticancer activity carried out using the SRB assay against the Colon cancer Cellline HCT-15 and the breast cancer cellline MCF7. Cytotoxicity activity was evaluated using the sulforhodamine B (SRB) assay and the experimental compounds investigated were solubilized in the appropriate solvent to prepare a stock of 1×10^{-2} concentration [21]. At the time of the experiment, four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µL of these different dilutions of compounds were added to the appropriate microtiter wells that already contain 90 µL of medium, resulting in the final concentrations of compounds required [22]. After the addition of compounds, the plates were incubated under standard conditions for 48 hours and the assay was completed by the addition of cold trichloroacetic acid (TCA). Cells were fixed in-situ by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% trichloroacetic acid) and incubated for 60 minutes at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. Sulforhodamine B solution (50 µL) at 0.4% (w/v) in 1% acetic acid to each of the wells and the plates were incubated for 20 minutes at room temperature. After staining, the unbound dye was recovered and the residual dye was

removed by washing five times with 1% acetic acid. Plates were air-dried. The bound stain was subsequently eluted with a 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with a reference wavelength of 690 nm [23].

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of the average absorbance of the test well to the average absorbance of the control wells ×100. Using the six absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)], the percentage growth was calculated at each of the investigated compounds concentration levels. The dose response parameters were calculated for each test article. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i-T_z)/(C-T_z)] \times 100 = GI_{50}$, which is the compounds concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during compounds incubation. The concentration of the investigated compounds that resulted in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of investigated compounds resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [24] $(T_i-T_z)/T_z] \times 100 = LC_{50}$ values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested [25].

2.4. Antituberculosis activity

Preparation of inoculum: The isolates grown in Lowenstein Jensen medium (LJ) were subcultured in Middle Brook 7H9 broth supple maintained with OADC (oleic acid, albumin, dextrose, and catalase) at 37 °C for 14-21 days. The antimycobacterial activities of the pyridine derivatives were evaluated against the M. bovis strain by maintaining them in Middle Brook 7H9 medium for approximately 14-21 days. The bacterial suspension was homogenized by vortex shakeup and the turbidity was adjusted in agreement with tube which is the scales of McFarland no 1 (3.2×10^6 cfu/mL). The inoculum was prepared by diluting the bacterial suspension in the pro portion of 1:20 in Middle Brook 7H9 broth medium. This diluted suspension (100 µL) was used to inoculate each well of the plate [26].

The *Mycobacterium bovis* stains were collected from the City Hospital Ahmedabad, Gujrat, India. The Resazurin microtiter assay (REMA) was used to test the antimycobacterial activity of the pyridine derivatives. Resazurin ® was used as a marker of cellular viability or growth inhibition in 96 microplates (wells) to perform the susceptibility test. Middle Brook 7H9 broth supplemented with OADC was used to dilute working solutions of the compounds tested, resulting in final sample concentrations ranging from 1, 2, 5, 10 mg/mL. As a positive control, Rifampicin® was dissolved in DMSO, and as a negative control, drug-free media containing strain suspensions were used. All testing wells and drug/extract-free control wells received 100 microliters of Middle Brook 7H9 broth and the test inoculum [27,28]. The first well of each row was then filled with 100 microliters of testing compounds solutions, from which two-fold dilution series were created using the microplate column. Each concentration of the extract was tested twice. After that, each microplate was sealed with parafilm and incubated for five to seven days at 37 °C in a standard atmosphere. 25 µL of resazurin 0.02% w/v was added to each well after the incubation period, and then the wells were re-incubated for 24 hours at 37 °C to develop color. All synthesized derivatives of pyridine were tested for their antimycobacterial activity against the *M. bovis* strain by maintaining on Middle Brook 7H9 at concentration 1, 2, 5, 10 m /mL compared to standard Isoniazid [29].

3. Results and discussion

3.1. Synthesis

Our work planned to synthesize of pyridine based azomethine derivatives 3a-h derivatives *via* the reaction of substituted 3-aminopyridine with substituted *ortho*-hydroxy benzaldehydes in ethyl alcohol, as described in Scheme 1 on treatment substituted 3-aminopyridine in water with in ethanol furnished the corresponding azomethine derivatives with loss of water molecules. The analytical analysis and spectral data evidenced the structure of compound 3a-h. The IR spectra of compounds 3a-h revealed an absorption band at 3260-3672 cm^{-1} due to the OH group, bands at 1575-1641 cm^{-1} corresponding to C=N groups, 1424-1477 cm^{-1} due to C=C aromatic, O-CH=CH_2 group attached to pyridine shows an IR band about 1084 cm^{-1}. ^{1}H NMR spectrum compounds 3a-h showed singlet signals at δ 3.38-3.37 ppm appear due to -C*H*$_2$=C-, δ 3.67-3.63 ppm appear doublet, due to -CH_3, δ 6.04-5.96 ppm singlet appear due to -OH, δ 6.97-6.94 ppm due to C=CH, δ 7.23-7.53 ppm peak due to aromatic protons, δ 10.92-10.90 ppm due to N=CH, groups, δ 8.02-8.00 ppm peak due to pyridine ring. ^{13}C NMR spectra of compounds 3a-h exhibited characteristic signals corresponding to various functional groups. A resonance at δ 56.55 ppm was attributed to the *C*H_2-O moiety, while the signal at δ 39.52 ppm corresponded to the two methyl groups. The signals observed at δ 110.20, 116.52, 116.83, and 129.25 ppm were assigned to the carbons of the pyridine ring. Furthermore, singlet signals at δ 137.45, 130.00, 143.45, and 149.00 ppm were associated with aromatic nuclei, indicating the presence of conjugated systems within the synthesized compounds.

3.2. In vitro anticancer activity

The anticancer activity of pyridine derivatives against the human colon cancer cell line HCT-15 using the sulforhodamine B assay assay at four different concentration levels. All results against human colon cancer cell line HCT-15 are available also in experimental values (Table 1). One of the biggest obstacles to cancer treatment is overcoming multidrug resistance (MDR) [30]. In this regard, compounds linked to Schiff bases have attracted a lot of attention in recent decades. Schiff bases are a promising field of study for potential colon cancer treatments because research indicates that they can have a significant anticancer effect on colon cancer cells, possibly inhibiting their growth and inducing apoptosis through various mechanisms [31]. However, more research is required to fully understand their efficacy and safety in humans. Rajput *et al.* reported that imine-based phenolic monoterpenoid derivatives show excellent effects on the HCT-15 colon cancer cell line with a remarkable GI_{50} value [32]. The 3d derivative has the lowest GI_{50} values, which are comparable with the standard drug molecule Adriamycin. Colon cancer cell growth inhibited by compounds containing imine and hydrazine linkages by blocking active infected sites. The results obtained show that seven derivatives based on pyridine have mild potency against the MCF-7 cell line. The total growths of inhibition at the 50% concentration level for compound 3d show excellent inhibition at each concentration. The GI_{50} for compound 3d is 22.5 µg/mL, which is comparable to the slandered drug molecule Adriamycin ®. The remaining all compounds show negligible inhibition against HCT-15 cell line shown in Table 2.

Table 1. Cytotoxicity effects (% control growth) of compounds 3a-h against the human colon cancer cell line HCT-15.

Compounds	Drug concentrations (µg/mL)			
	10	20	40	80
3a	114.0	124.8	126.5	120.9
3b	113.6	124.8	131.2	131.7
3c	120.2	118.9	128.0	131.9
3d	66.9	45.0	31.0	27.7
3e	128.1	123.3	111.8	99.8
3f	114.0	124.8	126.5	120.8
3g	113.6	124.8	125.1	131.9
3h	110.2	111.4	114.3	118.5
Adriamycin	5.5	2.0	1.4	0.7

Table 2. GI_{50}, TGI, and LC_{50} of compounds 3a-h against the human colon cancer cell line HCT-15 *.

Compounds	LC_{50} (µM)	TGI (µM)	GI_{50} (µM)
3a	NE	NE	>80
3b	NE	NE	>80
3c	>80	>80	>80
3d	>80	>80	22.5
3e	>80	>80	>80
3f	>80	>80	>80
3g	>80	>80	>80
3h	>80	>80	>80
Adriamycin	<10	<10	<10

* GI_{50}: Median growth inhibition; TGI: Total growth inhibition, LC_{50}: Median lethal concentration, NE: No effect.

Table 3. Cytotoxicity effects (% control growth) of compounds 3a-h against the human breast cancer cell line MCF-7.

Compounds	Drug concentrations (µg/mL)			
	10	20	40	80
3a	98.73	104.73	104.77	102.40
3b	96.93	104.07	104.50	95.43
3c	46.57	49.47	48.83	43.63
3d	103.33	114.37	113.00	109.70
3e	99.10	105.13	102.97	102.73
3f	114.00	124.08	126.50	120.90
3g	111.07	124.77	125.10	131.90
3h	128.07	123.33	111.80	99.83
Adriamycin	5.47	2.03	1.53	0.77

Table 4. GI_{50}, TGI, and LC_{50} of compounds 3a-h against the human breast cancer cell line MCF-7 *.

Compounds	LC_{50} (µM)	TGI (µM)	GI_{50} (µM)
3a	NE	>80	>80
3b	NE	>80	>80
3c	NE	>80	<10
3d	NE	>80	>80
3e	NE	>80	>80
3f	NE	>80	>80
3g	NE	>80	>80
3h	NE	>80	>80
Adriamycin	<10	<10	<10

* GI_{50}: Median growth inhibition; TGI: Total growth inhibition, LC_{50}: Median lethal concentration, NE: No effect

The anticancer activity of the pyridine derivatives evaluated against the human breast cancer cell line MCF-7 was evaluated using a sulphorodamine blue assay at four different concentration levels. All results against breast cancer cell line MCF-7 are available in triplicate experimental values (Table 3). Earlier research demonstrated that functionalized imine compounds show moderate to excellent against human breast cancer cell line MCF-7 [33]. Faraj and co-workers reported two novel quinazoline syntheses and characterizations [33]. The anticancer activity of the Schiff bases against the human breast cancer cell line MCF-7 was examined. With an IC_{50} value of 6.246×10^{-6} and 5.910×10^{-6} mol/L, respectively, the Schiff base 1 and the Schiff base 2 showed a notable antiproliferative effect [34]. One of the biggest obstacles to cancer treatment is overcoming multidrug resistance (MDR). In this regard, compounds linked to Schiff bases have attracted a lot of attention in recent decades. Schiff bases are a promising field of study for potential colon cancer treatments because research indicates that they can have a significant anticancer effect on colon cancer cells, possibly inhibiting their growth and inducing apoptosis through various mechanisms. However, more research is required to fully understand their efficacy and safety in humans. Rajput *et al.* reported that imine based phenolic monoterpenoid derivatives shows excellent effects on the MCF-7 breast cancer cell line with remarkable effect. The results obtained show that the seven derivatives have mild potency against MCF-7 cell line [35]. The results obtained show that the seven derivatives have mild potency against MCF-7 cell line. The total growths of inhibition at 50% concentration level for compound 3c show excellent inhibition at each concentration. The GI_{50} for compound 3c is 10 µg/mL, which is comparable to the slandered drug molecule Adriamycin ®. The remaining compound shows negligible inhibition against the MCF-7 cell line shown in Table 4.

3.3. Antituberculosis activity

Most recent studies have shown that pyridine compounds and their derivatives have a wide range of pharmacological activities. Our understanding of how to create more potent pyridine-based medications to treat tuberculosis has advanced greatly as a result of the consideration of MIC values, structural alterations, such as the addition of particular substitutes [36,37]. A pyridine-based drug called isoniazid is used to treat tuberculosis (TB). It is prescribed as first-line therapy and is one of the primary medications used to treat tuberculosis [38].

Table 5. *In vitro* antimycobacterial activity of compounds 3a-h against *M. Bovis*.

Compound	% Inhibition against *M. Bovis* (1 mg/mL)
3a	13.85
3b	6.48
3c	81.58
3d	22.19
3e	6.48
3f	6.37
3g	7.82
3h	6.28
Isoniazid	94.24

The obtained results show (Table 5) that pyridine based seven derivatives shows mild anti-TB against to *M. bovis*. The three-active site in a compound 3c is responsible for enhancing the % of inhibition at each concentration. The % inhibition of compound 3c is 81.58 which is comparable with a slandered isoniazid molecule. In compound 3c that has the highest percentage of inhibition at all concentrations is due to the effect of the amide functional group. Previous research showed the prolonged effect of the amide group on TB stains [39]. The % of inhibition for each compound against *M. Bovis* is showing considerable effect. In other compounds such as compound 3a, 3c, and 3d that have a progressive mode of inhibition against *M. Bovis*. The remaining compounds show negligible inhibition at all concentrations. The observed antimycobacterial activity against *M. Bovis* was unaffected by the type of substituent (R) or pyridine-based azomethine scaffolds.

4. Conclusions

Some new pyridine-based heterocyclic compounds 3a-h containing azomethine functional group were reported. These recently synthesized compounds were thoroughly described by spectrum analyses that perfectly matched the designated structures. Several of the compounds produced were tested using the sulforhodamine B assay against the human breast cancer cell line MCF-7, the human colon cancer cell line HCT-15, and antimycobacterial activity against the strain of *M. bovis*. On comparison of the newly produced compounds to the reference molecule, it was discovered that most of them exhibited antimycobacterial and anticancer activity.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered to.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Vasant Bhagwan Jagrut; Methodology: Sanjay Kotalwar; Software: Gautam Prabhakar Sadawarte; Validation: Sanjay Kotalwar; Formal Analysis: Amol Diliprao Kale; Investigation: Sanjay Kotalwar; Resources: Rajendra Prahlad Phase; Data Curation: Gautam Prabhakar Sadawarte; Writing - Original Draft: Sanjay Kotalwar; Writing and editing: Vasant Bhagwan Jagrut; Funding acquisition: Sanjay Kotalwar; Supervision: Vasant Bhagwan Jagrut.

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