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Synthesis, characterization, and biological activities of substituted pyridine-based azomethine scaffolds

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RESEARCH ARTICLE

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ABSTRACT

The present research work describes the synthesis of a new series of heterocyclic compounds, namely, pyridine-based azomethine scaffolds. A total of eight derivatives were prepared, purified, and characterized by analytical methods such as ^{1}H NMR, ^{13}C NMR, and IR spectroscopic techniques. All compounds were used to investigate their alpha-amylase inhibition activity. We have also reported antimicrobial activity using a micro broth dilution assay, with microbial strains *Pseudomonas aeruginosa* (NCIM 5031), *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NCIM 2699), *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902), and *Aspergillus flavus* (NCIM 549). Finally, we report the antioxidant activity of the synthesized derivatives using a DPPH free radical assay.

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1. Introduction

Pyridine nuclei have been reported to be found in a large number of naturally occurring compounds. Among nitrogen-based heterocycles, the compounds and content of pyridine scaffolds are valued for their biological, medicinal, optical, chemical, and physical properties [1]. One of the heterocycles used most frequently in the field of drug design is pyridine-based ring systems [2]. This is mainly due to their significant impact on pharmacological activity, which has resulted in the discovery of several broad-spectrum therapeutic agents [3]. Pyridine is added to ethyl alcohol as a solvent to render it intoxicating. Numerous biological activities of pyridine have been discovered, including those that are antiviral, anti-tubercular, anticancer, antibacterial, and antidiabetic. In this review, we have covered some more recent biological activities, including antidotes, antioxidants, antileishmanial, antichagasic, and antithrombin [4]. The pyridine core and its derivatives have shown broad biological effects, such as antimycobacterial [5,6], anti-inflammatory [7,8], analgesic activities [9], antibacterial activity against pathogenic bacterial strains (*S. aureus* and *E. coli*) [10], anticancer (leukemia, breast cancer and idiopathic pulmonary fibrosis) [11], antihypertensive in the treatment of angina pectoris [12], antihistamine [13], anticholinergic, antiulcerative [14], anti-HIV [15], antiviral agents [16-20], antidiabetic [21], and antioxidant potency [22,23].

Nicotinic and isoniazid derivatives are important classes of compounds based on pyridine having pharmacological properties such as *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* [24,25], *S. aureus* [26-28], anticancer against human cancer cells with potent cytotoxicity [29], anti-fungal activities *in vitro* against dimorphic fungus, *Histoplasma capsulatum* and var. *capsulatum* [30], potential multi-target profiles for the treatment of Alzheimer's disease [31]. Furthermore, Sadawarte *et al.* reported that thiopyridine derivatives have promising antidiabetic activity; recently, many more investigations reported hybrid pyridine nuclei in type I and type II diabetic research, on the basis of this vast literature, we have selected substituted scaffolds based on pyridine nuclei for the present investigation [32]. We designed and synthesized a series of new 3-azomethine hybrid derivatives 3a-h and characterized them using IR, ^{1}H NMR, ^{13}C NMR, and MS techniques,

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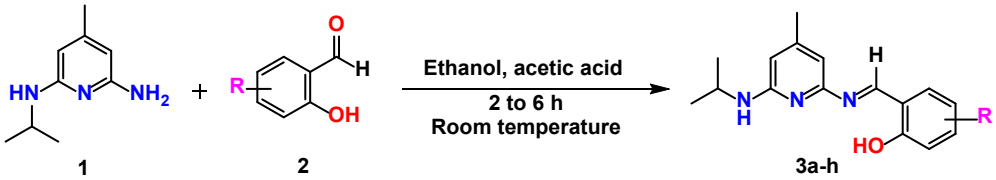
Table 1. Optimization for the synthesis of pyridine-based scaffolds.

Compound	R	Time required for synthesis, h
3a	H	4
3b	6-CH_3	6
3c	6-Br	3
3d	4-F	3
3e	4-Cl	2
3f	3,4-Cl	2
3g	3-Cl, 4-CH_3	4
3h	4-CF_3	4



Scheme 1. Synthesis of compounds 3a-h.

and to explore their alpha-amylase inhibition and antimicrobial activities.

2. Experimental

2.1. Instrumentation

The melting points were determined using the Tanco PLT-276 Delux Model melting temperature apparatus. The IR spectra were measured as KBr pellets by using a Shimadzu double beam infrared spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on an Advanced Bruker Neo AM 500 MHz spectrometer at room temperature in $CDCl_3$ solution using tetramethyl silane (TMS) as an internal standard reference. Chemical shifts were expressed in delta (ppm) downfield from TMS, and the coupling constants were in Hertz (Hz). LC-MS spectra were run on a Q-TOF Micromass Spectrometer (ESI-MS) at 70 eV.

2.2. Synthesis

We purchased high-purity chemicals from LOBA and Merck. *N*^{2}-isopropyl-4-methylpyridine-2,6-diamine was solved in 5 mL of ethanol and later in this solution was added a solution of the particular different substituted aldehyde (3a: 2-hydroxybenzaldehyde; 3b: 2-hydroxy-3-methylbenzaldehyde; 3c: 2-hydroxy-3-bromobenzaldehyde; 3d: 2-hydroxy-5-Fluorobenzaldehyde; 3e: 2-hydroxy-5-chlorobenzaldehyde; 3f: 2-hydroxy-5,6-dichlorobenzaldehyde; 3g: 2-hydroxy-5-methyl-6-chloro benzaldehyde; 3h: 2-hydroxy-5-trifluoromethylbenzaldehyde) in 5 mL of ethanol. A catalytic amount of glacial acetic acid (3 to 4 drops) was added. The resulting reaction mixture received was stirred at room temperature for 2-6 hours. The solvent was removed under reduced pressure, and the residue was then washed with water. Finally, the resulting solid product was collected and recrystallized from the solution of the ethanol: water mixture solution (80:20, *v*:*v*) to obtain the desired compound 3a-h (Scheme 1, Table 1).

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl) phenol (**3a**): Color: Pale yellow. Yield: 78%. M.p.: 102-104 °C. FT-IR (KBr, ν, cm): 3277 (O-H), 3277 (N-H), 2547 (C-H), 1610 (C=N), 1475, 1463 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.261 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.27 (s, 3H, CH_3), 2.86-2.91 (m, 1H, CH), 6.88-6.89 (d, 2H, *J* = 7.2 Hz, ArH), 7.10-7.12 (dd, 2H, *J* = 7.2 Hz, Py), 7.55-7.58 (t, 2H, *J* = 7.2 Hz, Ar-H), 7.70-7.71 (s, 1H, CH), 13.04-13.07 (s(b), 1H, H-N), 13.30-13.33 (s (b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 16.21, 24.21, 35.6, 76.82, 77.06, 77.27, 77.32, 115.01, 119.63, 123.20, 126.87, 131.06, 136.22, 152.09, 155.30. MS (ESI, *m/z*): 269.23 [M]$^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-6-methylphenol (**3b**): Color: Brown. Yield: 72 %. M.p.: 98-100 °C. FT-IR (KBr, ν, cm): 3292 (O-H), 2919 (N-H), 2567 (C-H), 1612 (C=N), 1469, 1390 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.24-1.26 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.27 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.84-3.86 (m, 1H, CH), 6.80-6.81 (d, 2H, *J* = 7.2 Hz, ArH), 7.10-7.12 (dd, 2H, *J* = 7.2 Hz, Py), 7.52-7.54 (t, 1H, *J* = 7.2 Hz, ArH), 7.71-7.72 (s, 1H, CH), 13.04-13.07 (s (b), 1H, H-N), 13.30-13.33 (s(b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 16.18, 24.19, 29.32, 32.82, 110.03, 115.91, 125.28, 133.40, 152.85, 154.48. MS (ESI, *m/z*): 282.26 [M]$^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-6-bromophenol (**3c**): Color: Red. Yield: 80 %. M.p.: 106-108 °C. FT-IR (KBr, ν, cm): 3169 (O-H), 2953 (N-H), 2767 (C-H), 1612 (C=N), 1483, 1384 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.22-1.26 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.23 (s, 3H, CH_3), 2.87-2.89 (m, 1H, CH), 7.22-7.24 (s, 1H, *J* = 7.2 Hz, Py), 7.50-7.51 (s, 1H, *J* = 7.2 Hz, Py), 7.70-7.72 (s, 1H, *J* = 7.2 Hz, ArH), 7.83-7.85 (d, 2H, *J* = 7.2 Hz, ArH), 7.92-7.96 (s, 1H, CH), 13.04-13.06 (s(b), 1H, NH), 13.40-13.43 (s(b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 16.13, 23.86, 32.26, 110.47, 116.89, 122.63, 126.60, 132.71, 135.28, 151.25, 153.90. MS (ESI, *m/z*): 349.00 [M]$^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-4-fluorophenol (**3d**): Color: Yellow. Yield: 80 %. M.p.: 100-102 °C. FT-IR (KBr, ν, cm): 3288 (O-H), 2947 (N-H), 2713 (C-H), 1610 (C=N), 1467, 1368 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.24-1.26 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.23 (s, 3H, CH_3), 2.80-2.81 (m, 1H, CH), 7.13-7.15 (s, 2H, *J* = 7.2 Hz, Py), 7.43-7.44 (s, 1H, *J* = 7.2 Hz, ArH), 7.61-7.62 (s, 1H, *J* = 7.2 Hz, ArH), 7.69-7.70 (s, 1H, *J* = 7.2 Hz, ArH), 7.70-7.72 (s, 1H, CH), 13.07-13.09 (s(b), 2H, H-N, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 15.06, 24.95, 32.80, 101.45, 112.99, 115.39, 118.06, 122.82, 125.32, 131.21, 133.40, 154.46, 154.46. MS (ESI, *m/z*): 286.00 [M]$^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-4-chlorophenol (**3e**): Color: Yellow. Yield: 82%. M.p.: 104-106 °C. FT-IR (KBr, ν, cm): 3273 (O-H), 2945 (N-H), 2738 (C-H), 1616 (C=N), 1467, 1309 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.26-1.28 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.24 (s, 3H, CH_3), 2.86-2.89 (m, 1H, CH), 7.12-7.15 (s, 1H, *J* = 7.2 Hz, Py), 7.30-7.32 (s, 1H, *J* = 7.2 Hz, Py), 7.66-7.69 (s, 2H, *J* = 7.2 Hz, ArH), 7.72 - 7.74 (s, 1H, *J* = 7.2 Hz, ArH), 7.74-7.77 (s, 1H, CH), 13.22-13.24 (s(b), 2H, H-N, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 15.78, 23.26, 32.72, 110.79, 119.32, 120.02, 123.22, 125.29, 126.80, 131.55, 136.35, 153.57, 154.62. MS (ESI, *m/z*): 302.00 [M]$^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-3,4-dichlorophenol (**3f**): Color: Creamy. Yield: 80 %. M.p.: 108-110 °C. FT-IR (KBr, ν, cm): 3267 (O-H), 2954 (N-H), 2627 (C-H), 1610 (C=N), 1463, 1377 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.25-1.27(d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.24 (s, 3H, CH_3), 2.88-

2.89 (m, 1H, CH), 7.16-7.18 (s, 1H, *J* = 7.2 Hz, Py), 7.73-7.75 (s, 1H, *J* = 7.2 Hz, Py), 7.83-7.87 (d, 2H, *J* = 7.2 Hz, ArH), 7.91-7.93 (s, 1H, CH), 12.75-12.78 (s(b), 1H, H-N), 13.40-13.41 (s(b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 15.76, 23.94, 32.69, 110.50, 121.06, 125.39, 131.64, 138.45, 154.59, 154.54. MS (ESI, *m/z*): 338.24 [M].

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-3-chloro-4-methyl phenol (**3g**): Color: Dark brown. Yield: 74 %. M.p.: 100-102 °C. FT-IR (KBr, ν, cm): 3304 (O-H), 2954 (N-H), 2721 (C-H), 1610 (C=N), 1405, 1304 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.23-2.24 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.24 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.61-2.63 (m, 1H, CH), 7.44-7.45 (s, 2H, *J* = 7.2 Hz, ArH + Py), 7.76-7.78 (s, 1H, *J* = 7.2 Hz, Py), 7.78-7.79 (s, 1H, *J* = 7.2 Hz, ArH), 7.81-7.82 (s, 1H, CH), 13.00-13.01 (s(b), 1H, H-N), 13.23-13.24 (s(b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 10.11, 20.01, 32.71, 111.02, 124.47, 125.07, 133.20, 139.04, 152.16, 154.43. MS (ESI, *m/z*): 316.00 $[M]^+$.

2-((6-(Isopropylamino)-4-methylpyridin-2-ylimino) methyl)-4-(trifluoromethyl)phenol (**3h**): Color: Creamy. Yield: 80%. M.p.: 102-104 °C. FT-IR (KBr, ν, cm): 3394 (O-H), 3061 (N-H), 2881 (C-H), 1625 (C=N), 1433, 1334 (C=C). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.25-2.26 (d, 6H, *J* = 6.8 Hz, 2×CH_3), 2.33-2.34 (s, 3H, CH_3), 2.81-2.82 (m, 1H, CH), 7.27-7.29 (s, 1H, *J* = 7.2 Hz, Py), 7.70-7.71 (s, 1H, *J* = 7.2 Hz, Py), 7.86-7.87 (s, 1H, *J* = 7.2 Hz, ArH), 7.87- 7.89 (s, 1H, *J* = 7.2 Hz, ArH), 8.01-8.04 (s, 1H, *J* = 7.2 Hz, ArH), 8.10-8.11 (s, 1H, CH), 13.01-13.02 (s(b), 1H, H-N), 13.24-13.25 (s(b), 1H, H-O). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 16.24, 23.95, 32.01, 112.03, 121.22, 124.32, 133.35, 139.66, 152.52, 154.55. MS (ESI, *m/z*): 336.01 $[M]^+$.

2.3. Biological activities

2.3.1. Evaluation of α-amylase inhibitory activity

α-Amylase inhibition assay was performed using a method previously reported in the literature [33]. 250 µL of total solutions of compounds having various concentrations were placed in different hard glass tubes and 250 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing α-amylase solution was added. All solutions were pre-incubated at 25 °C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.9) were added at time intervals and then again incubated at 25 °C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure as described above, replacing the compounds with water. The concentrations of the samples that resulted in 50% inhibition of enzyme activity (IC_{50}) were graphically determined.

2.3.2. Antimicrobial activity

In vitro antimicrobial activities of the derivatives were determined using different microorganisms by microbroth dilution assay [34]. Microbial strains, *Pseudomonas aeruginosa* (NCIM 5031), *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NCIM 2699), *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902), *Aspergillus flavus* (NCIM 549) were obtained from the National Chemical Laboratory, Pune, India. The bacteria were maintained in nutrient broth (NB) and the fungal strains were maintained in Sabouraud dextrose broth at 37 °C.

2.3.2.1. Preparation of inoculums

For bacteria, the bacterial strains used as inoculums were grown at 37 ° C to obtain optical density 0.6 at 600 nm. Colony forming units (CFU) were counted using the serial plate dilution method and bacterial counts were adjusted to 1×10^5 - 1×10^6 CFU/mL for susceptibility testing.

For the fungus, the fungal inoculums were prepared from cultures grown 10 days in potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 mL of distilled water and the conidia were scraped using a sterile spatula. The spore density of each fungus was adjusted with a spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 1×10^5 spores/mL.

Micro-Broth dilution assay - The determination of the minimum inhibitory concentration (MIC) was carried out using the micro-broth dilution method. According to the NCCLS guidelines. The test was carried out in 96-well culture plates (Hi-media). The compounds were dissolved in dimethyl sulfoxide to produce eight different concentrations, *viz*. 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL in the wells by a twofold dilution method. The negative control was prepared using dimethyl sulfoxide, and the same concentrations of tetracycline for bacteria and amphotericin B for the fungus were used as positive control. The 96 well plates were incubated for 24 h and 48 h at 37 °C for bacteria and fungus, respectively. The lowest concentration of each compound that inhibited visual growth was considered the MIC of that respective compound. All such experiments were repeated three times.

2.3.3. Antioxidant activity, DPPH assay

The antioxidant activity of the compound and the standard was taken on the basis of the radical scavenging effect of the stable DPPH free radical. 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical that can accept an electron or hydrogen radical to become a stable dual magnetic molecule. Due to its odd electron, the alcoholic solution of DPPH shows a strong absorption band at λ_{max} = 517 nm. DPPH radicals react with suitable reactive agents, and then the electrons become paired off, and the solution loses color equally to the number of electrons taken up. The reduction of DPPH radicals can be observed by decreasing the absorbance at λ_{max} = 517 nm. 100 ppm DPPH solution was prepared in alcohol and 1.0 mL of this solution was added to dilutions of the compound solution in water at different concentrations (25, 50, 100, 250, 500 ppm). Thirty minutes later, the absorbance was measured at λ_{max} = 517 nm. Butylated hydroxytoluene was used as standard.

3. Results and discussion

3.1. Synthesis

The *N*^{2}-isopropyl-4-methylpyridine-2,6-diamine used in this study was prepared by direct reduction of the nitro group of pyridine with iron in HCl solution following the standard procedure given in the reference [21,35]. Pyridine derivatives (3a-h) were synthesized in a single step method. First, substituted pyridines were reacted with benzaldehyde molecules in ethanol using glacial acetic acid as a catalyst. A total of eight derivatives were prepared using the same protocol. Depending on the substitution benzaldehyde, the reaction time is varied for each derivative. Compounds 3a-h were synthesized according to the method given in the experimental section and their structures were confirmed with the characterization data provided in the experimental section. The pure compounds obtained were then examined to determine their biological activities.

Table 2. α-Amylase inhibition activity of compounds **3a-h** at various concentrations with acarbose standard.

Compounds / Concentration	% Inhibition at different concentration			
	25 µL	50 µL	100 µL	250 µL
3a	12.00	16.40	20.20	20.80
3b	36.92	40.20	44.05	50.30
3c	8.20	12.10	12.90	16.20
3d	38.20	42.85	50.44	58.18
3e	23.20	26.80	32.40	24.80
3f	50.00	50.80	58.60	62.10
3g	4.20	10.50	12.20	12.90
3h	10.00	14.20	26.50	30.50
Acarbose	62.90	68.70	74.00	82.00

Table 3. Antimicrobial activity of compounds **3a-h**.

Compounds	Minimum inhibitory concentration each microbial species (mg/mL)						
	P. aeruginosa	*E. coli*	*S. typhi*	*B. subtilis*	*A. niger*	*A. fumigatus*	*A. flavus*
3a	2.5	2.5	2.5	5.0	2	2.5	2
3b	2.5	2.5	2.5	0.156	1.25	2.5	2.5
3c	5.0	5.0	5.0	0.312	1.25	5.0	2.5
3d	5.0	5.0	2.5	0.156	1.00	5.0	2.5
3e	10.5	5.0	2.5	5.0	1.25	2	2.5
3f	10.5	5.0	5.0	5.0	5.0	10.5	2.5
3g	5.0	5.0	5.0	5.0	5.0	5.0	2.5
3h	10.0	10.0	5.0	2.5	2.5	5.0	2.5
Tetracycline	0.00125	0.01	0.01	0.00125	-	-	-
Amphotericin B	-	-	-	-	0.00125	0.000156	0.000156

Table 4. Antioxidant activity of compounds 3a-f at various concentrations.

Compounds / Concentrations	% Inhibition at different concentration				
	25 ppm	50 ppm	100 ppm	250 ppm	500 ppm
3a	40.12	44.58	48.6	56.70	65.74
3b	36.92	40.20	44.05	50.30	55.56
3c	55.98	60.50	70.12	76.20	80.00
3d	38.20	42.85	50.44	58.18	64.80
3e	37.12	40.01	44.08	55.20	60.12
3f	50.00	58.00	60.00	76.00	80.00
3g	40.80	45.20	48.98	57.20	66.14
3h	32.50	38.20	40.20	45.50	48.20
Butylated hydroxytoluene	62.90	68.70	74.00	82.00	86.00

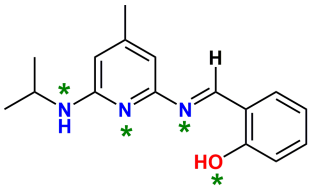


Figure 1. Possible active sites for free radical generation.

3.2. Biological activities

3.2.1. Evaluation of α-amylase inhibitory activity

α-Amylase inhibition activities of pyridine-based derivatives were carried out using α-amylase inhibition assay that shows inhibition at various concentrations (Table 2). Compounds **3b**, **3d**, **3e**, and **3f** had shown comparable α-inhibition at 25, 50, 100, and 250 µL, when compared to the standard acarbose molecule, while compounds **3a**, **3c,** and **3g** had lower inhibition at all concentrations. Theoretically α-amylase can be inhibited by NH-C=N-C=C-N-C=C-OH cavity, but the obtained result shows the effect of substituent on the inhibition of α-amylase. Compounds **3a**, **3c**, **3g** and **3h** that have bulky substituents on the aromatic nucleus can create a barrier in the protein cavity for inhibition. For this reason, compounds **3a**, **3c**, **3g**, and **3h** show low inhibition at all concentrations. Compounds **3b**, **3d**, **3e**, and **3f** show considerable inhibition at all concentrations due to perfect inhibition of the protein cavity. Among all derivatives, **3d** and **3f** show excellent inhibition at all concentrations which are comparable to the standard molecule, acarbose.

3.2.2 Antimicrobial activity

Different classes of microorganisms were selected from different habitats for antimicrobial activity. The microbial strains *Pseudomonas aeruginosa* (NCIM 5031), *Escherichia coli* (NCIM 2065), *Salmonella typhi* (NCIM 2501), exhibited a minimum inhibitory concentration (MIC) of 0.01 and 0.00125 mg/mL, respectively, for the standard antibacterial agent tetracycline. Whereas MICs of 0.00125, 0.000156, and 0.000156 mg/mL were shown by amphotericin B against *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902) and *Aspergillus flavus* (NCIM 549), respectively. The minimum inhibitory concentration 2.5 mg/mL was shown in derivatives **3a** and **3b** for Gram-ve bacteria. *E. Coli.* by compounds **3a**, **3b, 3d, 3e** and **3j** for *S. Typhi*, compounds **3b** and **3d** exhibited 0.156 mg/mL MIC for Gram +ve bacteria *Bacillus subtilis.* Compound **3f** showed a MIC of 0.625 for the pathogenic plant fungus *A. niger* in antifungal tests. The derivatives 3a and 3b showed 2.5 mg/mL MIC for *A. fumigatus* while seven derivatives exhibited 2.5 mg/mL MIC against *A. flavus.* From the results (Table 3), it can be concluded that compound **3f** has broad-spectrum antifungal and antibacterial activities, and compound **3f** has more antifungal activity. In contrast, the

activity of this compound is much less (MIC levels are very high) than that of the standard tetracycline and amphotericin B.

3.2.3. Antioxidant activity, DPPH assay

All substituted pyridine-based azomethine scaffolds had shown remarkable antioxidant activity at concentrations of 25, 50, 100, 250, and 500 ppm (Table 4). The entire compound with four active sites (Figure 1) from which radicals may be generated after its interaction with DPPH and which exhibits considerable radical scavenging activity over DPPH at lower concentrations. As the concentration of the DPPH molecule increases to 100, 250, and 500 ppm, the radical scavenging activity increases. Compounds **3a**, **3b**, **3d**, **3e**, **3g**, and **3h** have comparable activity at higher concentrations due to the temperate reactive substitution on the phenol ring. In contrast, compounds **3c** and **3f** have notable activity at higher concentrations due to reactive substitution on the phenol ring.

4. Conclusions

In summary, we present a simple synthetic method for preparing azomethine derivatives and characterizing them through various spectroscopic methods. A series of novel pyridine-based compounds labeled 3a-h were created, which incorporate an azomethine functional group. These compounds were synthesized using N^2-isopropyl-4-methylpyridine-2,6-diamine as building blocks. Comprehensive spectral analyses (IR, ^{1}H NMR, and ^{13}C NMR) confirmed the structures of these newly synthesized compounds. Subsequently, their anti-bacterial, antifungal, antioxidant and α-amylase inhibitory properties against Gram-positive bacteria, Gram-negative bacteria, and fungi were evaluated. The results revealed that many of the newly developed compounds exhibited significant activity in these areas compared to standard commercial drugs.

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Disclosure statement

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Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Vasant Bhagwan Jagrut; Methodology: Gautam Prabhakar Sadawarte; Software: Jamatsing Darbarsing Rajput; Validation: Amol Diliprao Kale; Formal Analysis: Jamatsing Darbarsing Rajput; Investigation: Amol Diliprao Kale; Resources: Rajendra Pralhadrao Phase; Data Curation: Rajendra Pralhadrao Phase; Writing - Original Draft: Gautam Prabhakar Sadawarte; Writing - Review and Editing: Gautam Prabhakar Sadawarte; Supervision: Vasant Bhagwan Jagrut.

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