



A convenient synthesis and biological activities of *N*-(pyridin-3-ylmethylene) benzohydrazides by the condensation of nicotinaldehydes with benzohydrazides

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Series of *N*-(pyridine-3-ylmethylene)benzohydrazides **3a-y** have been prepared by the condensation of nicotinaldehydes **1a-e** with benzohydrazides **2a-e** in the presence of glacial AcOH in ethanol at room temperature. Total twenty five compounds have been prepared and confirmed based on spectral data. The compounds have been evaluated for anti-microbial, free radical scavenging (DPPH, ABTS⁺) and α -glucosidase inhibitory activities. Compound **3h** has shown potent anti-fungal activity. Compounds **3f-g** and **3j** have shown potent ABTS⁺ free radical scavenging activity. Compound **3d** has shown potent anti-hyperglycemic activity.

Keywords: *N*-Pyridinylmethylenebenzohydrazides, nicotinaldehydes, benzohydrazides, anti-microbial activity, anti-hyperglycemic activity

Chloronicotinaldehydes are the important heterocyclic compounds and have potential applications in the areas of pharmaceutical and agrochemicals¹⁻⁶. Pyridine heterocyclic compounds have been achieved through C-C bond forming reactions such as Sonogashira⁷ and Suzuki-Miyaura coupling reactions⁸ by replacing the chloro group of the pyridine positioned at C-2. The replacement of chlorine with N-, O-, and S-containing nucleophiles also provides the novel heterocyclic compounds⁹. 2-Chloropyridine containing bio-active heterocyclic compounds¹⁰⁻¹² such as 1,3,4-oxadiazoles, flavones and dihydropyrazoles are well reported in the literature.

Benzohydrazides and their derivatives are promising organic compounds bearing -CO, -NH and -NH₂ functionalities¹³⁻¹⁵. Benzohydrazides have important applications in pharmaceutical and analytical chemistry^{16,17}. Heterocyclic compounds with benzohydrazide moiety have displayed various biological activities^{18,19} such as anti-leishmanial, anti-inflammatory, anti-cancer, anti-mycobacterial and anti-tumor.

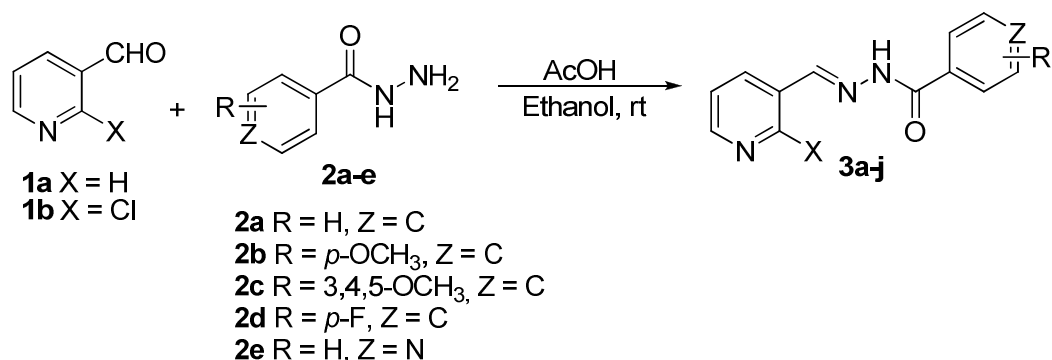
As part of our ongoing research on 2-chloronicotinaldehydes, we have reported useful heterocyclic compounds²⁰⁻²³. Recently, we have shown 2*H*-chromenylmethylene benzohydrazides as

potential anti-microbial agents²⁴. The present manuscript describes the preparation of *N*-(pyridin-3-ylmethylene) benzohydrazides and biological screening of anti-microbial, free radical scavenging (DPPH, ABTS⁺) and α -glucosidase inhibitory activities.

Results and Discussion

The preparation of *N*-(pyridin-3-ylmethylene) benzohydrazides **3a-e** depicted in Scheme I. In an initial experiment, we have chosen nicotinaldehyde **1a** as the starting substrate to prepare the target compound. Nicotinaldehyde **1a** (1.0 equiv.) was stirred with benzohydrazide **2a** (1.0 equiv.) in glacial AcOH (0.2 mL) in ethyl alcohol (2 mL) at RT. The reaction proceeded smoothly and provided *N*-(pyridin-3-ylmethylene)benzohydrazide **3a** as colourless solid in 91% yield. This result was encouraged us to carry out the reaction of **1a** with substituted benzohydrazides **2b-d** and Isoniazid **2e** under above reaction conditions. All these reactions proceeded smoothly and provided the target compounds **3b-j** (Table I). All the compounds are unknown and characterized by spectral data (see SI).

Having achieved *N*-pyridinylmethylene benzohydrazides **3a-j** and then we have planned to prepare the required starting materials to make another set of target



Scheme I

Table I — Preparation of *N*-(pyridin-3-ylmethylene)benzohydrazides **3a-y**^a

Entry	Compd	X	Z	R
1	3a	H	C	H
2	3b	H	C	4-OCH ₃
3	3c	H	C	3,4,5-OCH ₃
4	3d	H	C	4-F
5	3e	H	N	H
6	3f	Cl	C	H
7	3g	Cl	C	4-OCH ₃
8	3h	Cl	C	3,4,5-OCH ₃
9	3i	Cl	C	4-F
10	3j	Cl	N	H
11	3k	2-Piperidinyl	C	H
12	3l	2-Piperidinyl	C	4-OCH ₃
13	3m	2-Piperidinyl	C	3,4,5-OCH ₃
14	3n	2-Piperidinyl	C	4-F
15	3o	2-Piperidinyl	N	H
16	3p	2-Morpholinyl	C	H
17	3q	2-Morpholinyl	C	4-OCH ₃
18	3r	2-Morpholinyl	C	3,4,5-OCH ₃
19	3s	2-Morpholinyl	C	4-F
20	3t	2-Morpholinyl	N	H
21	3u	2-Thio-morpholinyl	C	H
22	3v	2-Thio-morpholinyl	C	4-OCH ₃
23	3w	2-Thio-morpholinyl	C	3,4,5-OCH ₃
24	3x	2-Thio-morpholinyl	C	4-F
25	3y	2-Thio-morpholinyl	N	H

^a Isolated yields

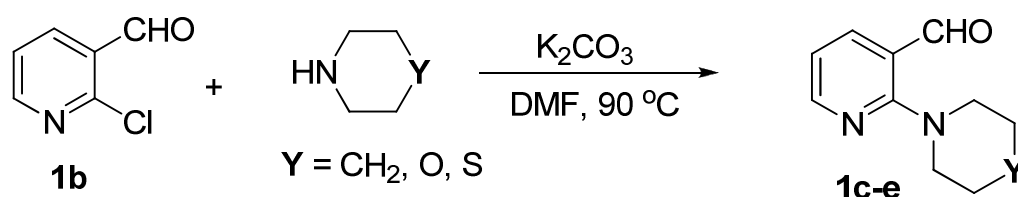
compounds. The starting materials were prepared by replacing the chloro group with moieties such as piperidine **1c**, morpholine **1d** and thio-morpholine **1e** as per the Scheme II. 2-Chloronicotinaldehyde **1b** was reacted with **1c-e** in the presence of K₂CO₃ in dry DMF at 90°C and furnished the corresponding nicotinaldehydes **1c-e**.

Scheme III describes the preparation of target compounds *N*-((2-substituted pyridinyl)methylene) benzohydrazides **3k-y**. Nicotinaldehydes **1c-e** (1.0 equiv.) were stirred with benzohydrazides **2a-e** (1.0

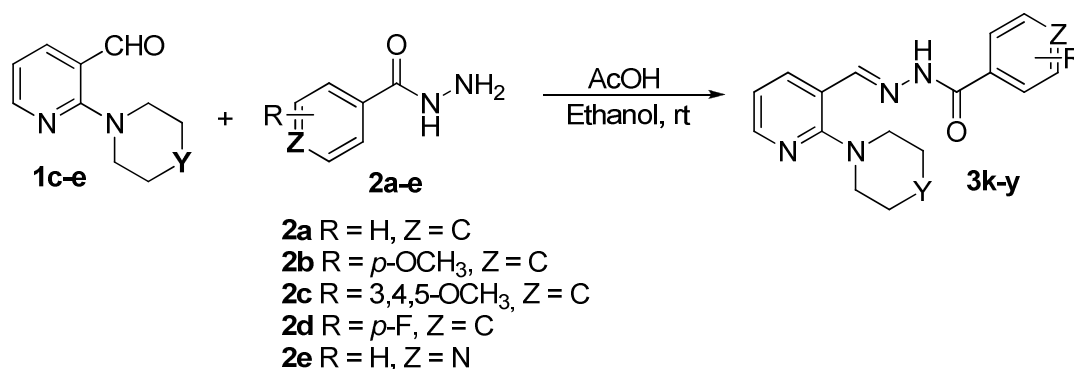
equiv.) in the presence of glacial AcOH (0.2 mL) in ethyl alcohol (2 mL) at RT. All these reactions underwent smoothly and provided the target compounds **3k-y** (Table I). All the prepared compounds **3a-y** are unknown and characterized by spectral data (see SI).

Biology

Thus prepared compounds **3a-y** were tested for anti-microbial, free radical scavenging (DPPH, ABTS⁺) and anti-hyperglycaemic (rat intestinal α -glucosidase) activities^{25,26} and the results are presented below.



Scheme II



Scheme III

Anti-microbial activity

The anti-microbial activity of compounds **3a-y** tested against two Gram-positive organisms (*Bacillus subtilis*; *Staphylococcus epidermis*) and two Gram-negative organisms (*Pseudomonas aeruginosa*; *Escherichia coli*) by agar well diffusion method and compared with the Streptomycin. The compounds data and zone of inhibition (mm) values were presented in Table II²⁵. Analysis of anti-bacterial potential of the tested compounds revealed that total twelve compounds have been displayed activity against tested gram positive and three compounds displayed against tested gram negative bacterial strains.

The compounds **3a**, **3k-l** and **3u-x** have shown the moderate anti-bacterial activity against *B. subtilis* and compounds **3b**, **3f-g**, **3n** and **3v** displayed moderate activity against *S. epidermis*. The compounds **3e**, **3h** and **3p** have shown moderate anti-bacterial activity against *E. coli*. The structure activity relationship of the compounds revealed that the compounds **3a**, **3v** (methoxy group present on benzohydrazide with thio-morpholine on pyridyl) and **3x** (fluoro group present on benzohydrazide with thio-morpholine on pyridyl) have shown moderate activity against *B. subtilis*. The compounds **3f** are having chloro substitution on pyridyl and compound **3g** having additional methoxy substitution present on phenyl shown the moderate activity against *S. epidermis*. The compound having fluoro group present on phenylhydrazide and

piperidine substitution at 2nd position on pyridyl **3n** (10 mm) displayed better anti-bacterial activity against *S. epidermis* when compared to compound **3i** having chloro substitution.

The anti-fungal activities of the target compounds **3a-y** were tested against *Candida albicans* and fluconazole was used as standard drug (Table II). The compounds **3h** has shown potent and **3i**, **3p** and **3v** have shown moderate anti-fungal activity. The structure activity relationship of the compounds revealed that the compounds **3h** having trimethoxy group present on benzohydrazide with chloro on pyridyl have shown potent activity when compared to mono methoxy compound **3g**. The compound **3p** was having morpholine substitution on pyridyl and compound **3v** having thio-morpholine present on pyridyl and methoxy present on phenyl shown the moderate activity.

DPPH, ABTS⁺ free radical scavenging activity

The free radical scavenging (DPPH and ABTS⁺) activity²⁶ of target compounds **3a-y** along with the standard drugs are presented in Table III. None of the compounds displayed DPPH free radical scavenging activity. Interestingly, compounds **3f-g** (SC₅₀ 1.32, 1.35 µg/mL) and **3j** (SC₅₀ 1.54 µg/mL) have shown potent and significant ABTS⁺ free radical scavenging activity in present series of compounds in comparison with Trolox (SC₅₀ 1.25 µg/mL).

Table II — Anti-microbial activity profile of compounds **3a-y**

Compd	Anti-bacterial				Anti-fungal
	Gram-positive		Gram-negative		<i>C. albicans</i>
	<i>B. subtilis</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	
3a	7	—	—	—	—
3b	—	6	—	—	—
3c	—	—	—	—	—
3d	—	—	—	—	—
3e	—	—	—	6	—
3f	—	7	—	—	—
3g	—	7	—	6	—
3h	—	—	—	—	18
3i	—	—	—	—	6
3j	—	—	—	—	—
3k	5	—	—	—	—
3l	6	—	—	—	—
3m	—	—	—	—	—
3n	—	10	—	—	—
3o	—	—	—	—	—
3p	—	—	—	7	9
3q	—	—	—	—	—
3r	—	—	—	—	—
3s	—	—	—	—	—
3t	—	—	—	—	—
3u	5	—	—	—	—
3v	8	7	—	—	8
3w	6	—	—	—	—
3x	7	—	—	—	—
3y	—	—	—	—	—
Control	0	0	0	0	0
Streptomycin	19	13	13	16	—
Fluconazole	—	—	—	—	22 (0.12, 0.25)

B. subtilis: Bacillus subtilis, *S. epidermidis*: Staphylococcus epidermidis, *P. aeruginosa*: Pseudomonas aeruginosa, *E. coli*: Escherichia coli, *C. albicans*: Candida albicans. The presented values represent the zone of inhibition in millimeter (mm) on agar plate against the represented microbial strains.

Anti-hyperglycemic activity

Table III represents the anti-hyperglycaemic (rat intestinal α -glucosidase) activity²⁶ and their IC₅₀ values of target compounds **3a-y** along with the standard drug Acarbose. Only one compound **3d** (IC₅₀ 3.02 μ g/mL) having fluoro substitution present on benzohydrazide moiety displayed potent anti-hyperglycemic activity in comparison with Acarbose (IC₅₀ 2.69 μ g/mL).

Experimental Section

All the chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA), AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F₂₅₄ (mesh); spots were

visualized under UV light. Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectrum was recorded with a Thermo Nicolet Nexus 670 FT spectrometer. ¹H and ¹³C NMR spectra were recorded on Bruker Avance 300, 400 and 500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESI-MS obtained on Quatro Micro spectrometer.

General experimental procedure for the preparation of (*E*)-*N'*-(pyridin-3-ylmethylene) benzohydrazides, **3a-y**

Glacial acetic acid (0.2 mL) was added to a stirred solution of nicotinaldehyde **1a** (1.0 mmol), benzohydrazide **2a** (1.0 mmol) in absolute ethanol (3 mL) at RT and the contents were stirred at the same

Table III — DPPH, ABTS⁺ and α -Glucosidase inhibitory activity profile of compounds **3a-y**

Compd	DPPH % Inhibition 25 μ g/mL (SC ₅₀ μ g /mL)	ABTS ⁺ % Inhibition 20 μ g /mL (SC ₅₀ μ g /mL)	α -AGI % Inhibition 20 μ g/mL (IC ₅₀ μ g /mL)
3a	4.42±0.04	16.55±0.32	1.78±0.00
3b	6.97±0.16	40.72±0.63	ND
3c	ND	11.19±2.21	0.31±0.00
3d	1.61±0.04	19.80±1.11	90.31±0.00 (3.02)
3e	5.88±0.57	14.32±1.90	8.65±0.35
3f	26.96±0.41	97.65±0.16 (1.32)	ND
3g	46.06±1.10	97.32±0.00 (1.35)	ND
3h	11.33±0.24	31.99±0.32	1.59±0.26
3i	6.94±0.93	31.88±0.47	4.35±0.17
3j	31.34±0.73	93.96±0.32 (1.54)	ND
3k	1.18±0.00	17.79±1.42	ND
3l	0.80±0.04	47.87±0.00	ND
3m	ND	51.90±1.27	ND
3n	1.00±0.00	43.06±1.11	ND
3o	4.59±0.93	26.17±0.32	ND
3p	21.25±1.78	27.52±0.00	4.23±0.00
3q	ND	38.14±2.06	ND
3r	0.20±0.00	24.05±0.16	ND
3s	3.81±0.24	38.81±0.17	ND
3t	5.88±0.08	28.86±0.32	ND
3u	0.86±0.61	21.25±0.00	ND
3v	ND	26.73±1.11	ND
3w	4.27±0.00	12.08±0.63	ND
3x	5.65±0.16	30.31±0.79	ND
3y	8.12±0.17	25.28±0.00	ND
Ascorbic acid	84.08±0.12	—	—
Trolox	—	98.88±0.00 (1.25)	—
Acarbose	—	—	91.17±0.00 (2.69)

DPPH: 1,1-Diphenyl-2-picrylhydrazyl; ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid);

AGI: α -glucosidase inhibition; ND-Not detected

temperature. After completion of the reaction (TLC, 1 h), the reaction mixture was filtered through Whatman filter paper and compound was recrystallized from ethanol to afford (*E*)-*N'*-(pyridin-3-ylmethylene)benzohydrazide **3a** as colourless solid. The compounds **3b-y** were prepared by the reaction of nicotinaldehydes **1a-b** with benzohydrazides **2a-e** under above conditions. All the prepare compounds are unknown and well characterized by spectral data.

(*E*)-*N'*-(Pyridin-3-ylmethylene)benzohydrazide, 3a: Yield 91%. Colourless solid. m.p. 192-194°C. FT-IR (KBr): 3187, 3021, 1675, 1549, 1419, 1278, 1140, 1024 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.03 (s, 1H), 8.87 (s, 1H), 8.62 (d, *J* = 3.8 Hz, 1H), 8.52 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.4 Hz, 2H), 7.76-7.24 (m, 4H); ¹³C NMR (101 MHz, DMSO-*d*₆): δ 163.74, 151.19, 149.21, 145.53, 133.98, 133.71, 132.39, 130.74, 129.01, 128.15, 124.52; ESI-MS: *m/z* 226 [M+H]⁺.

(*E*)-4-Methoxy-*N'*-(pyridin-3-ylmethylene)benzohydrazide, 3b: Yield 90%. Colourless solid. m.p. 165-167°C. FT-IR (KBr): 3187, 3008, 1667, 1606, 1512, 1312, 1259, 1177, 1028 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.90 (s, 1H), 8.85 (s, 1H), 8.61 (dd, *J* = 4.6, 1.2 Hz, 1H), 8.51 (s, 1H), 8.14 (d, *J* = 6.7 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 2H), 7.49 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆): δ 163.11, 162.60, 151.05, 149.13, 144.86, 133.84, 130.85, 130.10, 125.70, 124.49, 114.24, 55.93; ESI-MS: *m/z* 256 [M+H]⁺.

(*E*)-3,4,5-Trimethoxy-*N'*-(pyridin-3-ylmethylene)benzohydrazide, 3c: Yield 89%. Colourless solid. m.p. 172-174°C. FT-IR (KBr): 3455, 3198, 2939, 2832, 1665, 1587, 1504, 1467, 1329, 1129 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.88 (s, 1H), 8.87 (s, 1H), 8.63 (d, *J* = 3.8 Hz, 1H), 8.53 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H), 7.50 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.25 (s,

2H), 3.87 (s, 6H), 3.74 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.13, 153.20, 151.20, 149.22, 145.48, 141.04, 133.91, 130.73, 128.79, 124.53, 105.76, 79.66, 60.63, 56.60; ESI-MS: m/z 316 [M+H] $^+$.

(E)-4-Fluoro-N'-(pyridin-3-ylmethylene)

benzohydrazide, 3d: Yield 89%. Colourless solid. m.p. 212-214°C. FT-IR (KBr): 3193, 3003, 1678, 1567, 1505, 1416, 1374, 1306, 1235, 1143 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 12.05 (s, 1H), 8.87 (s, 1H), 8.63 (d, $J = 3.8$ Hz, 1H), 8.51 (s, 1H), 8.16 (d, $J = 7.7$ Hz, 1H), 8.07-7.81 (m, 2H), 7.50 (dd, $J = 7.4$, 4.9 Hz, 1H), 7.39 (t, $J = 8.7$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 165.94, 163.46, 162.65, 151.23, 149.25, 145.62, 133.94, 130.94, 130.85, 130.68, 130.18, 124.50, 116.11, 115.89; ESI-MS: m/z 244 [M+H] $^+$.

(E)-N'-(Pyridin-3-ylmethylene)

isonicotinohydrazide, 3e: Yield 89%. Colourless solid. m.p. 232-234°C. FT-IR (KBr): 3183, 3001, 2846, 1682, 1568, 1416, 1293, 1150 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.24 (s, 1H), 8.89 (d, $J = 1.7$ Hz, 1H), 8.85-8.70 (m, 2H), 8.70-8.57 (m, 1H), 8.53 (s, 1H), 8.17 (ddd, $J = 8.7$, 5.2, 3.4 Hz, 1H), 7.98-7.70 (m, 2H), 7.50 (dt, $J = 33.0$, 16.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 161.73, 150.97, 150.34, 148.89, 146.30, 140.20, 133.60, 125.07, 124.03, 121.51; ESI-MS: m/z 227 [M+H] $^+$.

(E)-N'-(2-Chloropyridin-3-yl)methylene

benzohydrazide, 3f: Yield 93%. Colourless solid. m.p. 165-167°C. FT-IR (KBr): 3180, 3027, 1647, 1580, 1401, 1351, 1148, 1070 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.22 (s, 1H), 8.81 (s, 1H), 8.48 (d, $J = 2.9$ Hz, 1H), 8.43-8.30 (m, 1H), 7.95 (d, $J = 7.3$ Hz, 2H), 7.62 (d, $J = 7.1$ Hz, 1H), 7.55 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.70, 151.24, 149.86, 142.93, 136.22, 133.43, 132.55, 129.11, 129.03, 128.20, 124.30; ESI-MS: m/z 260 [M+H] $^+$.

(E)-N'-(2-Chloropyridin-3-yl)methylene-4-methoxybenzohydrazide, 3g: Yield 89%. Colourless solid. m.p. 168-170°C. FT-IR (KBr): 3416, 3227, 3051, 1607, 1565, 1401, 1177, 1061 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.10 (s, 1H), 8.78 (s, 1H), 8.47 (dd, $J = 4.6$, 1.9 Hz, 1H), 8.37 (d, $J = 6.1$ Hz, 1H), 7.94 (d, $J = 8.7$ Hz, 2H), 7.54 (dd, $J = 7.7$, 4.7 Hz, 1H), 7.08 (d, $J = 8.8$ Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.08, 162.73, 151.09, 149.76, 142.13, 136.13, 130.17, 129.23, 125.42, 124.29, 114.28, 55.95; ESI-MS: m/z 290 [M+H] $^+$.

(E)-N'-(2-Chloropyridin-3-yl)methylene-3,4,5-trimethoxybenzohydrazide, 3h: Yield 91%,

Colourless solid, m.p. 172-174°C. FT-IR (KBr): 3207, 2937, 2871, 1649, 1584, 1500, 1333, 1228, 1125, 1086, 1005 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 12.10 (s, 1H), 8.79 (s, 1H), 8.52-8.45 (m, 1H), 8.39 (d, $J = 7.2$ Hz, 1H), 7.56 (dd, $J = 7.7$, 4.7 Hz, 1H), 7.27 (s, 2H), 3.88 (s, 6H), 3.74 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 163.19, 153.22, 151.23, 149.80, 142.65, 141.28, 136.28, 129.13, 128.49, 124.32, 105.85, 60.64, 56.64; ESI-MS: m/z 350 [M+H] $^+$.

(E)-N'-(2-Chloropyridin-3-yl)methylene-4-

fluorobenzohydrazide, 3i: Yield 82%. Colourless solid. m.p. 179-181°C. FT-IR (KBr): 3186, 3037, 1657, 1551, 1400, 1282, 1058 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.23 (s, 1H), 8.79 (s, 1H), 8.48 (d, $J = 2.9$ Hz, 1H), 8.38 (d, $J = 7.5$ Hz, 1H), 8.10-7.96 (m, 2H), 7.55 (dd, $J = 7.5$, 4.7 Hz, 1H), 7.40 (t, $J = 8.7$ Hz, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 166.05, 163.56, 162.63, 151.27, 149.86, 143.02, 136.22, 130.96, 129.90, 129.05, 124.30, 116.05; ESI-MS: m/z 278 [M+H] $^+$.

(E)-N'-(2-Chloropyridin-3-

yl)methylene)isonicotinohydrazide, 3j: Yield 88%. Colourless solid. m.p. 184-186°C. FT-IR (KBr): 3172, 2990, 1681, 1555, 1405, 1286, 1149, 1060 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.41 (s, 1H), 8.82 (d, $J = 5.2$ Hz, 3H), 8.50 (dd, $J = 4.6$, 1.9 Hz, 1H), 8.39 (dd, $J = 7.8$, 1.9 Hz, 1H), 7.86 (dd, $J = 4.5$, 1.6 Hz, 2H), 7.57 (dd, $J = 7.7$, 4.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 162.29, 151.55, 150.90, 150.03, 144.23, 140.49, 136.38, 128.83, 124.38, 122.03; ESI-MS: m/z 261 [M+H] $^+$.

(E)-N'-(2-(Piperidin-1-yl)pyridin-3-

yl)methylene)benzohydrazide, 3k: Yield 83%. Colourless solid. m.p. 243-245°C. FT-IR (KBr): 3208, 3066, 2936, 2818, 1640, 1555, 1432, 1370, 1291, 1235 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.95 (s, 1H), 8.52 (s, 1H), 8.34-8.19 (m, 1H), 8.10 (d, $J = 6.2$ Hz, 1H), 7.91 (d, $J = 7.2$ Hz, 2H), 7.60 (d, $J = 7.2$ Hz, 1H), 7.54 (t, $J = 7.3$ Hz, 2H), 7.05 (dd, $J = 7.5$, 4.8 Hz, 1H), 3.23-2.77 (m, 4H), 1.70 (d, $J = 3.5$ Hz, 4H), 1.59-1.10 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.72, 162.13, 149.19, 145.25, 135.58, 133.98, 132.25, 128.97, 128.14, 120.64, 118.10, 52.57, 26.06, 24.39; ESI-MS: m/z 309 [M+H] $^+$.

(E)-4-Methoxy-N'-(2-(piperidin-1-yl)pyridin-3-

yl)methylene)benzohydrazide, 3l: Yield 92%. Colourless solid. m.p. 212-214°C. FT-IR (KBr): 3200, 2934, 2831, 1634, 1431, 1372, 1260, 1176, 1063 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.83

(s, 1H), 8.51 (s, 1H), 8.28 (dd, $J = 4.7, 1.8$ Hz, 1H), 8.08 (d, $J = 7.1$ Hz, 1H), 7.91 (m, 2H), 7.05 (m, 3H), 3.84 (s, 3H), 3.24-2.86 (m, 4H), 1.93-1.62 (m, 4H), 1.63-1.43 (m, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.12, 162.50, 162.05, 149.03, 144.63, 135.50, 130.07, 125.96, 120.79, 118.09, 114.20, 55.93, 52.55, 26.06, 24.40; ESI-MS: m/z 339 [M+H] $^+$.

(E)-3,4,5-Trimethoxy-*N'*-((2-(piperidin-1-yl)pyridin-3-yl)methylene)benzohydrazide, 3m: Yield 94%. Colourless solid. m.p. 224-226°C. FT-IR (KBr): 2936, 1645, 1582, 1561, 1502, 1431, 1334, 1237, 1127 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.81 (s, 1H), 8.53 (s, 1H), 8.29 (dd, $J = 4.6, 1.7$ Hz, 1H), 8.09 (d, $J = 6.4$ Hz, 1H), 7.21 (s, 2H), 7.05 (dt, $J = 16.6, 8.3$ Hz, 1H), 3.87 (s, 6H), 3.74 (s, 3H), 3.22-2.92 (m, 4H), 1.69 (d, $J = 3.5$ Hz, 4H), 1.59 (d, $J = 4.7$ Hz, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.33, 162.20, 153.20, 149.18, 145.28, 140.98, 135.68, 129.17, 120.61, 118.10, 105.83, 60.63, 56.65, 52.56, 26.01, 24.37; ESI-MS: m/z 399 [M+H] $^+$.

(E)-4-Fluoro-*N'*-((2-(piperidin-1-yl)pyridin-3-yl)methylene)benzohydrazide, 3n: Yield 87%. Colourless solid. m.p. 232-234°C. FT-IR (KBr): 3217, 2935, 1639, 1591, 1432, 1370, 1238, 1059 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.96 (s, 1H), 8.51 (s, 1H), 8.29 (dd, $J = 4.6, 1.6$ Hz, 1H), 8.18-8.03 (m, 1H), 7.99 (dd, $J = 8.5, 5.6$ Hz, 2H), 7.39 (t, $J = 8.8$ Hz, 2H), 7.05 (dd, $J = 7.5, 4.8$ Hz, 1H), 3.25-2.91 (m, 4H), 1.70 (d, $J = 3.4$ Hz, 4H), 1.59 (d, $J = 4.8$ Hz, 2H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 162.62, 162.14, 149.22, 145.35, 135.58, 130.92, 130.83, 130.43, 130.72, 120.58, 118.10, 116.32, 115.96, 52.57, 26.07, 24.39; ESI-MS: m/z 327 [M+H] $^+$.

(E)-*N'*-((2-(Piperidin-1-yl)pyridin-3-yl)methylene)isonicotinohydrazide, 3o: Yield 87%. Colourless solid. m.p. 242-244°C. FT-IR (KBr): 3193, 3062, 2934, 2814, 1646, 1578, 1430, 1369, 1236, 1066 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 12.17 (s, 1H), 8.80 (dd, $J = 4.5, 1.5$ Hz, 2H), 8.53 (s, 1H), 8.31 (dd, $J = 4.7, 1.9$ Hz, 1H), 8.11 (dd, $J = 7.6, 1.8$ Hz, 1H), 7.83 (dd, $J = 4.5, 1.5$ Hz, 2H), 7.07 (dd, $J = 7.6, 4.8$ Hz, 1H), 3.11 (d, $J = 5.3$ Hz, 4H), 1.70 (s, 4H), 1.59 (d, $J = 4.1$ Hz, 2H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 162.34, 150.82, 149.49, 146.48, 141.04, 135.74, 123.61, 122.06, 120.29, 118.12, 52.62, 26.07, 24.37; ESI-MS: m/z 310 [M+H] $^+$.

(E)-*N'*-((2-Morpholinopyridin-3-yl)methylene)benzohydrazide, 3p: Yield 85%. Colourless solid. m.p. 227-229°C. FT-IR (KBr): 3248, 2838, 1646, 1582, 1427, 1366, 1292, 1144 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.93 (s, 1H), 8.56 (s, 1H), 8.33

(dd, $J = 4.6, 1.6$ Hz, 1H), 8.14 (d, $J = 6.3$ Hz, 1H), 7.91 (d, $J = 7.2$ Hz, 2H), 7.58 (dt, $J = 26.9, 7.2$ Hz, 3H), 7.12 (dd, $J = 7.5, 4.8$ Hz, 1H), 3.82-3.76 (m, 4H), 3.16-3.09 (m, 4H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.22, 160.62, 148.76, 144.38, 135.35, 133.48, 131.75, 128.47, 127.62, 120.23, 118.22, 66.18, 51.25; ESI-MS: m/z 311 [M+H] $^+$.

(E)-4-Methoxy-*N'*-((2-Morpholinopyridin-3-yl)methylene)benzohydrazide, 3q: Yield 86%. Colourless solid. m.p. 201-203°C. FT-IR (KBr): 3265, 2850, 1640, 1430, 1363, 1261, 1117 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.81 (s, 1H), 8.55 (s, 1H), 8.32 (dd, $J = 4.7, 1.8$ Hz, 1H), 8.11 (s, 1H), 7.96-7.81 (m, 2H), 7.22-6.83 (m, 3H), 3.84 (s, 3H), 3.83-3.70 (m, 4H), 3.23-2.91 (m, 4H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.10, 162.53, 161.08, 149.14, 144.21, 135.79, 130.07, 125.95, 120.89, 118.77, 114.23, 66.69, 55.94, 51.75; ESI-MS: m/z 341 [M+H] $^+$.

(E)-3,4,5-Trimethoxy-*N'*-((2-morpholinopyridin-3-yl)methylene)benzohydrazide, 3r: Yield 84%. Colourless solid. m.p. 228-230°C. FT-IR (KBr): 3180, 3029, 2858, 1643, 1582, 1430, 1364, 1237, 1117 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.79 (s, 1H), 8.56 (s, 1H), 8.33 (d, $J = 3.3$ Hz, 1H), 8.13 (d, $J = 7.1$ Hz, 1H), 7.21 (s, 2H), 7.12 (d, $J = 2.5$ Hz, 1H), 3.87 (s, 6H), 3.82-3.76 (m, 4H), 3.74 (s, 3H), 3.14 (s, 4H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.37, 161.11, 153.23, 149.27, 144.89, 141.02, 136.02, 129.22, 120.74, 118.76, 105.85, 66.64, 60.64, 56.68, 51.75; ESI-MS: m/z 401 [M+H] $^+$.

(E)-4-Fluoro-*N'*-((2-morpholinopyridin-3-yl)methylene)benzohydrazide, 3s: Yield 82%. Colourless solid. m.p. 226-228°C. FT-IR (KBr): 3256, 2836, 1648, 1596, 1428, 1237, 1112 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 11.94 (s, 1H), 8.55 (s, 1H), 8.33 (d, $J = 4.8$ Hz, 1H), 8.13 (d, $J = 6.7$ Hz, 1H), 8.06-7.86 (m, 2H), 7.39 (t, $J = 8.7$ Hz, 2H), 7.12 (dd, $J = 7.4, 4.9$ Hz, 1H), 3.79 (d, $J = 4.1$ Hz, 4H), 3.13 (s, 4H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 162.63, 161.15, 149.32, 144.98, 135.89, 130.91, 130.62, 120.68, 118.76, 116.10, 115.89, 66.69, 51.77; ESI-MS: m/z 329 [M+H] $^+$.

(E)-*N'*-((2-Morpholinopyridin-3-yl)methylene)isonicotinohydrazide, 3t: Yield 81%. Colourless solid. m.p. 169-171°C. FT-IR (KBr): 3218, 3112, 1660, 1553, 1419, 1332, 1227 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 12.12 (s, 1H), 8.81 (dd, $J = 4.4, 1.6$ Hz, 2H), 8.57 (s, 1H), 8.34 (dd, $J = 4.8, 1.9$ Hz, 1H), 8.14 (dd, $J = 7.6, 1.8$ Hz, 1H), 7.82 (dd, $J = 4.4, 1.6$ Hz, 2H), 7.13 (dd, $J = 7.6, 4.8$ Hz, 1H), 3.91-3.54

(m, 4H), 3.25-2.89 (m, 4H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 162.17, 161.26, 150.86, 149.59, 146.12, 141.02, 136.06, 122.04, 120.40, 118.79, 66.69, 51.80; ESI-MS: m/z 312 [M+H] $^+$.

(E)-N'-((2-Thiomorpholinopyridin-3-yl)methylene) benzohydrazide, 3u: Yield 83%. Colourless solid. m.p. 223-225°C. FT-IR (KBr): 3209, 3066, 2833, 1641, 1579, 1430, 1368, 1292, 1068 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 11.94 (s, 1H), 8.53 (s, 1H), 8.39-8.24 (m, 1H), 8.14 (d, $J = 7.5$ Hz, 1H), 7.91 (d, $J = 7.2$ Hz, 2H), 7.61 (d, $J = 7.2$ Hz, 1H), 7.55 (t, $J = 7.4$ Hz, 2H), 7.12 (dd, $J = 7.5, 4.8$ Hz, 1H), 3.48-3.35 (m, 4H), 2.83 (dd, $J = 5.9, 3.6$ Hz, 4H); ^{13}C NMR (101 MHz, DMSO- d_6): δ 163.71, 162.00, 149.23, 144.82, 135.84, 133.97, 132.29, 129.00, 128.12, 121.09, 118.81, 53.83, 27.35; ESI-MS: m/z 309 [M+H] $^+$.

(E)-4-Methoxy-N'-((2-thiomorpholinopyridin-3-yl)methylene)benzohydrazide, 3v: Yield 83%. Colourless solid. m.p. 231-233°C. FT-IR (KBr): 3186, 2835, 1644, 1582, 1425, 1335, 1233, 1123, 1001 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 11.82 (s, 1H), 8.52 (s, 1H), 8.37-8.20 (m, 1H), 8.12 (d, $J = 7.1$ Hz, 1H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.10 (t, $J = 9.9$ Hz, 3H), 3.84 (s, 3H), 3.38 (m, 4H), 2.84 (m, 4H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 163.36, 162.54, 161.93, 149.09, 144.19, 135.76, 130.06, 125.94, 121.23, 118.81, 114.23, 55.94, 53.80, 27.33; ESI-MS: m/z 339 [M+H] $^+$.

(E)-3,4,5-Trimethoxy-N'-((2-thiomorpholinopyridin-3-yl)methylene)benzohydrazide, 3w: Yield 91%. Colourless solid. m.p. 207-209°C. FT-IR (KBr): 3182, 2833, 1645, 1586, 1428, 1333, 1238, 1127, 1001 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 11.82 (s, 1H), 8.53 (s, 1H), 8.32 (d, $J = 3.0$ Hz, 1H), 8.12 (d, $J = 6.8$ Hz, 1H), 7.21 (s, 2H), 7.12 (dd, $J = 7.5, 4.8$ Hz, 1H), 3.87 (s, 6H), 3.74 (s, 3H), 3.41 (s, 4H), 2.92-2.68 (m, 4H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 163.37, 162.93, 153.22, 149.23, 144.84, 141.61, 135.97, 129.23, 121.04, 118.80, 105.84, 60.64, 56.68, 53.79, 27.25; ESI-MS: m/z 417 [M+H] $^+$.

(E)-4-Fluoro-N'-((2-thiomorpholinopyridin-3-yl)methylene)benzohydrazide, 3x: Yield 92%. Colourless solid. m.p. 230-232°C. FT-IR (KBr): 3215, 2833, 1640, 1588, 1431, 1369, 1238, 1096 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 11.97 (s, 1H), 8.52 (s, 1H), 8.37-8.22 (m, 1H), 8.21-8.04 (m, 1H), 7.99 (dd, $J = 8.6, 5.6$ Hz, 2H), 7.40 (t, $J = 8.8$ Hz, 2H), 7.12 (dd, $J = 7.5, 4.8$ Hz, 1H), 3.69-3.36 (m, 4H), 2.83 (s, 4H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 162.61, 162.01, 149.28, 144.93, 135.86, 130.90,

130.86, 121.03, 118.83, 116.11, 115.89, 53.83, 27.34; ESI-MS: m/z 345 [M+H] $^+$.

(E)-N'-((2-Thiomorpholinopyridin-3-yl)methylene) isonicotinohydrazide, 3y: Yield 85%. Colourless solid. m.p. 256-258°C. FT-IR (KBr): 3191, 3058, 2834, 1648, 1583, 1430, 1366, 1300, 1225, 1065 cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6): δ 12.15 (s, 1H), 8.81 (dd, $J = 4.5, 1.5$ Hz, 2H), 8.54 (s, 1H), 8.34 (dd, $J = 4.7, 1.8$ Hz, 1H), 8.14 (dd, $J = 7.6, 1.8$ Hz, 1H), 7.82 (dd, $J = 4.5, 1.5$ Hz, 2H), 7.13 (dd, $J = 7.6, 4.8$ Hz, 1H), 3.40 (dd, $J = 6.0, 3.4$ Hz, 4H), 2.93-2.68 (m, 4H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 162.17, 162.12, 150.85, 149.54, 146.10, 141.01, 136.02, 122.03, 120.73, 118.83, 53.86, 27.34; ESI-MS: m/z 327 [M+H] $^+$.

Anti-bacterial activity

The anti-bacterial activity of prepared compounds were evaluated against two Gram-positive organisms (*Bacillus subtilis*; *Staphylococcus aureus*) and two Gram-negative organisms (*Pseudomonas aeruginosa*, *Escherichia coli*) by agar well diffusion method by using streptomycin as standard²³. The anti-fungal activity of the prepared compounds was evaluated against yeast (*Candida albicans*, MTCC 3017) and fluconazole was used as standard drug.

Zone of inhibition plate tests

Well plate method is followed for both the anti-bacterial and anti-fungal activities for measuring the zone of inhibitions²⁵. For anti-bacterial activity test strains used Gram positive and Gram negative in nutrient agar. For anti-fungal studies test strains used yeast and the medium used is potato dextrose agar. The synthesized compounds were used for activity studies and the concentration of each compound is 1.0 mg/mL along with standard and control. The media, petri dishes were autoclaved at 121 °C for 15 min. After sterilization the plates were poured with appropriate medium left over for 30 min for solidification, later the plates were inoculated with 60 μl of test inoculum using sterile cotton swabs. An 8 mm width size wells were made with sterile cork borer and in each well exactly 100 μl of sample were loaded. Control and standard also placed in separate wells. The plates were initially incubated for 20-30 min at 4 °C to allow the compounds to diffuse into the agar, and then subsequently incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. Zone diameters were expressed in mm using calibrated scale. Experiment was triplicate to minimize the deviations.

Determination of MIC and MBC

Minimum inhibition concentration (MIC) is the lowest concentration of an anti-microbial agent that will inhibit the visible growth of a microorganism. The MIC was determined using the tube dilution method. The compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 0.500 mg/mL (stock solution). The compounds having better anti-microbial activity were selected for the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) studies against all above microbial strains. The concentrations of test samples were serially diluted from 500 to 1.9 µg/mL and one tube without drug serves as control. All the tubes were inoculated with 1 mL of respective cultures having an OD of 0.2 (~ McFarland standard) and the tubes were incubated at 37 °C for 16 h. The turbidity of each tube is measured with respect to control tube. MIC values are defined as the lowest concentration of compound at which growth is completely inhibited. After incubation the culture from each tube was spread on nutrient agar plates to evaluate the MBC concentration. The concentration at which the cells are completely dead was defined as MBC.

DPPH scavenging activity

Assay for the scavenging of stable free radical based on DPPH [1,1-diphenyl-2-picrylhydrazyl] was done as reported earlier was performed²⁵. Briefly, in a 96-well micro plate, 25 µL of test sample dissolved in DMSO (1 mg/mL), 125 µL of 0.1 M tris-HCl buffer (pH 7.4) and 125 µL of 0.5 mM DPPH solution dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in dark condition for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as $(1-B/A) \times 100$ where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples.

ABTS⁺ free radical scavenging assay

Scavenging of the ABTS⁺ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was performed as described by Walker and Everette with suitable modifications²⁶. Briefly, 100 mL stock solution of ABTS⁺ (0.5 mM) was prepared by addition of 1 mL potassium persulfate (6.89 mM PBS, pH 8.0). The mixture was stored in the dark for 16 h. Test compounds were dissolved in DMSO (5mg/mL).

Primary screening was done by mixing 10 µL of test compound in 100 µL of methanol followed by 190 µL of ABTS⁺ in a 96-well microplate. Absorbance of decolorized ABTS⁺ was measured at 734 nm after 15 min incubation in the dark on a BioTek synergy⁴ multi-mode microplate reader. For each test sample a separate blank sample (devoid of ABTS⁺) was used for background subtraction. The percentage of ABTS⁺ scavenging was calculated applying following formula:

$$\% \text{ ABTS}^+ \text{ scavenging} = [(Absorbance_{\text{control}} - Absorbance_{\text{test}}) / Absorbance_{\text{control}} \times 100].$$

Various serial dilutions of active compounds were prepared and tested for determination of SC₅₀ values. Suitable regression analysis was applied for calculation of SC₅₀.

Anti-hyperglycemic activity

α-Glucosidase inhibitory activity was determined as per earlier reported method²⁶. Rat intestinal acetone powder in normal saline (100:1; w/v) was sonicated properly and the supernatant was used as a source of crude intestinal α-glucosidase after centrifugation. In brief, 20 µL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 µL of 100 mM-phosphate buffer (pH 6.8) in 96-well microplate and incubated with 50 µL of crude intestinal α-glucosidase for 5 min before 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in same buffer) was added. Release of *p*-nitrophenol was measured at 405 nm spectrophotometrically (Spectra Max plus 384), Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 µL of buffer. Control sample contained 10 µL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as $(1-B/A) \times 100$ where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples. For calculation of 50% enzyme inhibitory activity (IC₅₀) more than five dilutions of primary screening concentration (5 mg/mL DMSO solution) of test compounds were prepared. The IC₅₀ values were calculated applying logarithmic regression analysis.

Conclusions

In conclusion, series of *N'*-(pyridin-3-ylmethylene) benzohydrazides **3a-y** have been prepared by the condensation of nicotinaldehydes **1a-e** with

benzohydrazides **2a-e** in the presence of glacial AcOH in ethanol at RT. Compounds were evaluated for anti-microbial, free radical scavenging (DPPH, ABTS⁺) and α -glucosidase inhibitory activities. Compound **3h** identified as anti-fungal agent. Compounds **3f-g** and **3j** identified as potent ABTS⁺ free radical scavenging activity and compound **3d** identified as potent α -glucosidase inhibitor.

[Supplementary Information](#)

Supplementary information is available in the website <http://nopr.niscair.res.in/handle/123456789/60>.

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