Indian Journal of Chemistry
Vol. 59B, July 2020, pp. 991-1000

Condensation of nicotinaldehydes with phenylethanones: A convenient synthesis and biological activities of chalcones

S Ramya[a], K Sai Teja[a], K S Hariprasad[a], G Praveena[a], A Zehra[b], A K Tiwari[b], R S Prakasham[a] & B China Raju*[a]

[a] Department of Organic Synthesis & Process Chemistry
and
[b] Centre for Natural Products & Traditional Knowledge,
CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
E-mail: chinaraju@iict.res.in

Received 3 September 2019; accepted (revised) 22 January 2020

Claisen-Schmidt condensation of nicotinaldehydes **1a-e** with various phenylethanones **2a-d** in the presence of base at room temperature have provided chalcones **3a-t**. All the synthesized compounds have been evaluated for their anti-microbial, free-radical scavenging and α-glucosidase inhibitory activities. Compounds **3d** and **3h** have been identified as potent anti-fungal and moderate anti-bacterial agents. Compounds **3c**, **3h**, **3k-m** and **3q** have shown α-glucosidase inhibitory activity.

Keywords: Nicotinaldehydes, chalcones, anti-microbial activity, α-glucosidase inhibitory activity

Chalcones[1] are biologically active heterocyclic compounds and received considerable attention due to their simple structures with potential pharmacological activities such as anti-bacterial[2], anti-fungal[3], anti-inflammatory[4], anti-tumor[5], anti-oxidant[6], antitubercular[7], antimalarial[8], human monoamine oxidase (MAO)[9], NF*k*B inhibitors[10] and potential antiprotozoal derivatives[11].

2-Chloronicotinaldehydes are valuable compounds having chloro and formyl functional groups. As part of our research work on 2-chloronicotinaldehydes various heterocyclic compounds have been prepared such as Imidacloprid analogues[12], quinolines[13], 1,8-naphthyridines[14], *Baylis-Hillman* adducts[15], 2-chloro-5-methylpyridine-3-olefin derivatives[16], (*E*)-α,β-unsaturated esters and ketones[17], azlactones[18], 1*H*-1,2,3-triazolylbenzohydrazides[19], chromanones[20], and 1*H*-1,2,3-triazolylisonicotinohydrazides[21]. The present research work has been designed to prepare nicotinaldehydes based chalcone derivatives and evaluation of their anti-microbial, DPPH, $ABTS^{\cdot+}$ free radical scavenging and α-glucosidase inhibitory activities.

Results and Discussion

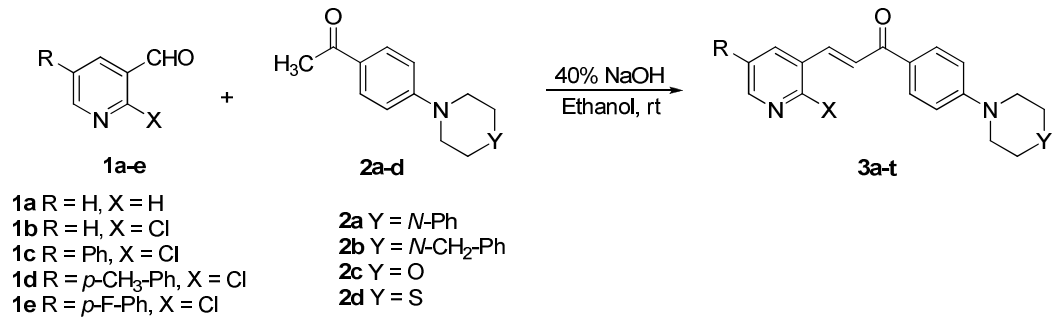
The preparations of target chalcone compounds **3a-t** have been depicted in Scheme I. The starting materials 2-chloronicotinaldehydes **1b-e** have been prepared as per our earlier reported method[22]. The substituted acetophenones such as 1-(4-morpholin ophenyl)ethanone **2a**, 1-(4-thiomorpholino phenyl)ethanone **2b**, 1-(4-(4-phenylpiperazin-1-yl)phenyl)ethanone **2c**, and 1-(4-(4-benzylpiperazin-1-yl)phenyl)ethanone **2d** have been prepared by the reaction of 1-(4-fluorophenyl)ethanone with 1-phenylpiperazine, 1-benzylpiperazine, morpholine and thiomorpholine in the presence of K_2CO_3 in DMF solvent at 110°C for 24 h. Claisen-Schmidt condensation of nicotinaldehydes **1a-e** with phenylethanones **2a-d** in the presence of 40% aq. sodium hydroxide in ethyl alcohol at room temperature afforded the target chalcone compounds **3a-t**. All the compounds are unknown and characterized by spectral data (Table I).

The chalcones **3a-t** have been evaluated for their biological activities such as anti-microbial[23], DPPH, $ABTS^{\cdot+}$ free radical scavenging and α-glucosidase inhibitory[24] and the results were discussed below.

Anti-microbial activity

All the compounds **3a-t** were tested for their anti-microbial activity against two Gram-positive organisms (*Bacillus megaterium*, *Staphylococcus aureus*) and two Gram-negative organisms (*Salmonella typhi and Escherichia coli*) by agar well

IICT Communication number: IICT/Pubs.2019/282



Scheme I

Table I — Preparation of nicotinaldehyde based chalcone derivatives **3a-t**

Entry	Compd	R	X	Y	Yield [a](%)
1	**3a**	H	H	*N*-C_6H_5	56
2	**3b**	H	H	*N*-$CH_2C_6H_5$	63
3	**3c**	H	H	O	67
4	**3d**	H	H	S	67
5	**3e**	H	Cl	*N*-C_6H_5	61
6	**3f**	H	Cl	*N*-$CH_2C_6H_5$	50
7	**3g**	H	Cl	O	56
8	**3h**	H	Cl	S	52
9	**3i**	C_6H_5	Cl	*N*-C_6H_5	55
10	**3j**	C_6H_5	Cl	*N*-$CH_2C_6H_5$	53
11	**3k**	C_6H_5	Cl	O	23
12	**3l**	C_6H_5	Cl	S	69
13	**3m**	4-$CH_3C_6H_5$	Cl	*N*-C_6H_5	62
14	**3n**	4-$CH_3C_6H_5$	Cl	*N*-$CH_2C_6H_5$	59
15	**3o**	4-$CH_3C_6H_5$	Cl	O	45
16	**3p**	4-$CH_3C_6H_5$	Cl	S	61
17	**3q**	4-F-C_6H_5	Cl	*N*-C_6H_5	42
18	**3r**	4-F-C_6H_5	Cl	*N*-$CH_2C_6H_5$	33
19	**3s**	4-F-C_6H_5	Cl	O	31
20	**3t**	4-F-C_6H_5	Cl	S	25

[a] Isolated yields

plate method and the results were presented in Table II. Two compounds **3d** and **3h** have shown significant anti-bacterial activity against tested gram positive (*B. megaterium*) and gram negative (*S. typhi*) bacterial strains when compared to standard drug. Compounds **3a-c**, **3e-g**, **3i-k** and **3m-n** have shown moderate anti-bacterial activity against *B. megaterium.* Only two compounds **3d** and **3h** have shown moderate anti-bacterial activity against *S. aureus*. Seven compounds **3c**, **3g**, **3k-l**, **3p** and **3r-t** have shown moderate anti-bacterial activity against *S. typhi*. Ten compounds **3a-h**, **3j** and **3m** have shown moderate anti-bacterial activity against *E. coli*.

The structure activity relationships of the compounds revealed that the chalcone compound **3d** (MIC 16, µg/mL) having thio-morpholine moiety without chloro substitution on pyridyl displayed moderate anti-microbial activity when compare to *N*-phenyl **3a**, *N*-benzyl **3b** and morpholine **3c**. The compound having thio-morpholine moiety **3h** (MIC 16, µg/mL) with chloro substitution on pyridyl displayed moderate anti-microbial activity when compared to *N*-phenyl **3i**, *N*-benzyl **3j** and morpholone **3k**. Further, the presence of phenyl **3i-l**, 4-methylphenyl **3m-p** and 4-fluorophenyl **3q-t** on pyridyl reduced the activity.

Anti-fungal activities of target compounds **3a-t** were tested against *Candida albicans* and fluconazole was used as standard drug (Table II). The compounds **3d** (MIC 8 µg/mL) and **3h** (MIC 8 µg/mL) were

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

	Anti-bacterial				Anti-fungal
	Gram-positive		Gram-negative		
Compd	*B. megaterium* (MIC)*	*S. aureus*	*S. typhi* (MIC)*	*E. coli*	*C. albicans* (MIC)*
3a	13	–	–	11	13
3b	14	–	–	12	–
3c	14	–	12	14	10
3d	16 (16)	12	14 (32)	14	14 (8)
3e	12	–	–	11	–
3f	12	–	–	12	–
3g	14	–	12	14	13
3h	15 (16)	12	18 (16)	12	14 (8)
3i	12	–	–	–	–
3j	12	–	–	12	–
3k	12	–	12	–	–
3l	–	–	13	–	11
3m	14	–	–	12	–
3n	12	–	–	–	–
3o	–	–	–	–	–
3p	–	–	13	–	13
3q	–	–	–	–	–
3r	–	–	13	–	–
3s	–	–	12	–	–
3t	–	–	13	–	13
Control	0	0	0	0	0
Streptomycin	27 (8)	20	20 (8)	22	–
Fluconazole	–	–	–	–	15 (8)

B. megaterium: Bacillus megaterium, *S. aureus*: Staphylococcus aureus, *S. typhi*: Salmonella typhi, *E. coli*: Escherichia coli, *C. albicans*: Candida albicans.
*MIC (µg/mL): Minimum inhibitory concentration.
The values represent the zone of inhibition in millimeter (mm) on agar plate against the represented microbial strains. The value in parentheses represents the MIC value (µg/mL).

shown potent anti-fungal activity in comparison with the standard drug (MIC 8 µg/mL). Compounds **3a, 3c, 3g, 3l, 3p** and **3t** have shown moderate anti-fungal activity. The results of anti-microbial activity of the compounds **3a-t** revealed that the thio-morpholine compounds **3d** and **3h** have shown both anti-bacterial and anti-fungal against when compared to morpholine, *N*-phenyl and *N*-benzyl.

Free radicals scavenging activity

The DPPH and $ABTS^{\cdot+}$ free radical scavenging activity (SC_{50} values) of nicotinaldehyde based chalcones (**3a-d**) and 2-chloronicotinaldehyde based chalcones (**3e-t**) are presented in Table III along with the standard drugs Ascorbic acid and Trolox. All these compounds have not shown promising free radical scavenging activity. However, compounds **3a** and **3i-t** have shown some DPPH free radical scavenging activity. However, compounds **3b-3h** could not detect the DPPH free radical scavenging activity. Whereas, compounds **3a-t** have shown some $ABTS^{\cdot+}$ free radical scavenging activity.

α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity of nicotinaldehyde based chalcones (**3a-d**) and 2-chloronicotinaldehyde based chalcones (**3e-t**) and their IC_{50} values presented in Table III along with the standard drug Acarbose. Total six compounds have shown α-glucosidase inhibitory activity in the present series of compounds among the synthesized 20 compounds. The compound having *N*-phenyl moiety on piperizine **3d** (IC_{50} 1.43 µg/mL) has shown α-glucosidase inhibitory activity when compared to morpholine **3a**, thio-morpholine **3b** and *N*-benzyl **3d** when compared to standard drug (IC_{50} 0.92 µg/mL). The thio-morpholine compound **3h** displayed α-glucosidase inhibitory activity (IC_{50} 2.86 µg/mL) when compared to *N*-phenyl **3e**, *N*-benzyl **3f**, and morpholine **3g**. The compounds morpholine (IC_{50} 1.39 µg/mL) and thio-morpholine (IC_{50} 1.33 µg/mL)

Table III — DPPH, $ABTS^{-+}$, α-glucosidase inhibitory activity profile of compounds 3a-t

Compd	DPPH % Inhibition 25 µg/mL (SC_{50} µg /mL)	$ABTS^{-+}$ % Inhibition 20 µg /mL (SC_{50} µg /mL)	α-GI % Inhibition 20 µg/mL (IC_{50} µg /mL)
3a	2.30 ± 0.17	44.96 ± 1.70	ND
3b	ND	8.80 ± 0.32	ND
3c	ND	6.99 ± 3.72	73.74 ± 0.49 (1.43)
3d	ND	8.72 ± 0.21	ND
3e	ND	15.71 ± 1.59	52.36 ± 0.85
3f	ND	16.84 ± 0.00	ND
3g	ND	9.70 ± 0.53	ND
3h	ND	9.55 ± 0.96	60.77 ± 1.57 (2.86)
3i	34.58 ± 0.13	16.54 ± 1.06	57.78 ± 1.18
3j	27.83 ± 1.96	2.86 ± 0.64	56.84 ± 0.24
3k	21.75 ± 0.00	10.98 ± 0.00	74.53 ± 0.62 (1.39)
3l	20.15 ± 0.08	3.61 ± 1.28	75.31 ± 0.49 (1.33)
3m	18.99 ± 0.19	46.32 ± 0.43	74.61 ± 0.54 (1.41)
3n	29.96 ± 0.40	8.05 ± 0.97	ND
3o	11.71 ± 0.21	13.16 ± 0.53	ND
3p	26.25 ± 0.86	8.12 ± 0.21	50.36 ± 0.62
3q	52.46 ± 0.21	13.61 ± 0.53	67.61 ± 1.36 (1.74)
3r	44.08 ± 0.00	7.07 ± 0.21	ND
3s	36.12 ± 0.04	3.53 ± 0.11	ND
3t	26.88 ± 0.08	6.77 ± 0.19	46.87 ± 0.21
Ascorbic acid	91.04 ± 0.02	–	–
Trolox	–	99.62 ± 0.32	–
Acarbose	–	–	81.95 ± 0.41 (0.92)

having phenyl substitution on pyridyl shown α-glucosidase inhibitory activity when compared to phenyl **3i** and *N*-benzyl **3j**. Further, the *N*-phenyl compounds **3m**, **3q** (IC_{50} 1.41, 1.74 µg/mL) having methyl and fluoro substitution on phenyl shown α-glucosidase inhibitory activity when compared to *N*-benzyl **3n**, **3r**, morpholine **3o**, **3s** and thio-morpholine **3p**, **3t**. Overall, in the present series of compounds, the compounds **3d**, **3h** displayed anti-microbial activity and compounds **3c**, **3h**, **3k-m** and **3q** shown α-glucosidase inhibitory activity.

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 F_{254} (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. ^{1}H NMR and ^{13}C 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 quarto micro spectrometer.

General experimental procedure for the preparation of chalcones 3a-t

Nicotinaldehyde **1a** (1.0 mmol) and 1-(4-morpholinophenyl)ethanone **2a** (1.20 mmol) were dissolved in ethanol (2 mL). Aqueous sodium hydroxide solution (40%, 0.3 mL) was added gradually to the reaction mixture at RT and the contents were stirred until the starting materials disappeared (TLC). After completion of the reaction, the solvent was removed under reduced pressure. Cold water was added to the residue, neutralized with cold acetic acid and the reaction mixture was extracted with ethyl acetate (2 × 20 mL). The organic layer was washed with brine (2 × 20 mL) and the layers were separated. The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The crude product was purified by column chromatography (EtOAc/hexane) gave yellow color solid **3a**. Similarly, compounds **3b-t** have been prepared by the reaction of nicotinaldehydes **1a-e** with acetophenones **2b-d** under optimized conditions. All the compounds are unknown and characterized by spectral data.

(*E*)-1-(4-(4-Phenylpiperazin-1-yl)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one, 3a: Yellow colored solid. m.p. 110-112°C. FT-IR (KBr): 3446, 2825, 1647, 1604, 1579, 1495, 1306, 1231, 1161, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.87 (s, 1H, aromatic), 8.61 (d, *J* = 3.7 Hz, 1H, aromatic), 8.03 (d, *J* = 8.9 Hz, 2H, aromatic), 7.94 (d, *J* = 7.9 Hz, 1H, aromatic), 7.77 (d, *J* = 15.7 Hz, 1H, CH), 7.63 (d, *J* = 15.7 Hz, 1H, CH), 7.35 (dd, *J* = 7.8, 4.8 Hz, 1H, aromatic), 7.31 (t, *J* = 7.9 Hz, 2H, aromatic), 7.00-6.96 (m, 4H, aromatic), 6.92 (t, *J* = 7.3 Hz, 1H, aromatic), 3.58-3.55 (m, 4H, $2CH_2$), 3.38-3.34 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.25, 154.09, 150.83, 150.50, 150.47, 149.55, 149.51, 139.22, 134.67, 130.80, 129.24, 127.91, 123.96, 120.33, 116.32, 113.59, 49.02, 47.25; ESI-MS: *m/z* 370 $[M+H]^+$; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{24}N_3O$ = 370.1919, found = 370.1923.

(*E*)-1-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one, 3b: Pale yellow solid. m.p. 181-183°C. FT-IR (KBr): 3422, 2936, 2815, 1652, 1608, 1588, 1347, 1230, 1193 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.85 (d, *J* = 1.3 Hz, 1H, aromatic), 8.60 (d, *J* = 3.6 Hz, 1H, aromatic), 7.99 (d, *J* = 9.0 Hz, 2H, aromatic), 7.93 (d, *J* = 8.0 Hz, 1H, aromatic), 7.76 (d, *J* = 15.7 Hz, 1H, CH), 7.62 (d, *J* = 15.7 Hz, 1H, CH), 7.35 (s, 2H, aromatic), 7.34 (t, *J* = 4.0 Hz, 3H, aromatic), 7.32-27 (m, 1H, aromatic), 6.90 (d, *J* = 9.0 Hz, 2H, aromatic), 3.57 (s, 2H, CH_2), 3.43-3.37 (m, 4H, $2CH_2$), 2.63-2.57 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.20, 154.28, 150.62, 149.74, 139.10, 137.68, 134.48, 131.0, 130.75, 129.12, 128.31, 127.55, 127.24, 123.90, 123.67, 113.36, 62.95, 52.65, 47.16; ESI-MS: *m/z* $[M+H]^+$ 484; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{25}H_{26}N_3O$ = 384.2076, found = 384.2073.

(*E*)-1-(4-Morpholinophenyl)-3-(pyridin-3-yl)prop-2-en-1-one, 3c: Pale yellow solid. m.p. 178-180°C. FT-IR (KBr): 2920, 2852, 1652, 1596, 1351, 1306, 1226, 1190, 1117, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.87 (s, 1H, aromatic), 8.62 (d, *J* = 3.9 Hz, 1H, aromatic), 8.05-7.99 (m, 2H, aromatic), 7.94 (dd, *J* = 7.9, 1.7 Hz, 1H, aromatic), 7.77 (d, *J* = 15.7 Hz, 1H, CH), 7.63 (d, *J* = 15.7 Hz, 1H, CH), 7.36 (dd, *J* = 7.9, 4.8 Hz, 1H, aromatic), 6.93 (d, *J* = 9.0 Hz, 2H, aromatic), 3.89-3.86 (m, 4H, $2CH_2$), 3.37-3.34 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.30, 154.30, 150.62, 149.67, 139.33, 134.57, 131.04, 130.71, 128.19, 123.77, 123.72, 113.32, 66.50, 47.33; ESI-MS: *m/z* $[M+H]^+$ 295; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{18}H_{19}N_2O_2$ = 295.1447, found = 295.1453.

(*E*)-3-(Pyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one, 3d: Pale yellow solid. m.p. 139-142°C. FT-IR (KBr): 2909, 1651, 1589, 1397, 1302, 1183, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.86 (d, *J* = 1.8 Hz, 1H, aromatic), 8.61 (dd, *J* = 4.8, 1.5 Hz, 1H, aromatic), 8.04-7.97 (m, 2H, aromatic), 7.94 (dt, *J* = 7.9, 1.8 Hz, 1H, aromatic), 7.77 (d, *J* = 15.7 Hz, 1H, CH), 7.62 (d, *J* = 15.7 Hz, 1H, CH), 7.36 (dd, *J* = 7.9, 4.8 Hz, 1H, aromatic), 6.90-6.83 (m, 2H, aromatic), 3.86-3.81 (m, 4H, $2CH_2$), 2.74-2.70 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.05, 152.98, 150.61, 149.68, 139.16, 134.51, 131.01, 127.28, 123.78, 123.67, 113.64, 50.23, 25.79; ESI-MS: *m/z* $[M+H]^+$ 411.

(*E*)-3-(2-Chloropyridin-3-yl)-1-(4-(4-phenylpiperazin-1-yl)phenyl)prop-2-en-1-one, 3e: Yellow coloured solid. m.p. 150-152°C. FT-IR (KBr): 2921, 2852, 1594, 1453, 1393, 1221, 1191 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.41 (dd, *J* = 4.7, 1.8 Hz, 1H, aromatic), 8.05 (s, 1H, aromatic), 8.04-8.02 (m, 1H, aromatic), 8.01 (s, 1H, aromatic), 8.00 (s, 1H, aromatic), 7.53 (d, *J* = 15.7 Hz, 1H, CH), 7.33 (d, *J* = 3.7 Hz, 1H, aromatic), 7.31 (d, *J* = 1.5 Hz, 1H, aromatic), 7.30 (d, *J* = 5.0 Hz, 1H, aromatic), 6.99 (d, *J* = 2.8 Hz, 2H, aromatic), 6.97 (d, *J* = 4.0 Hz, 2H, aromatic), 6.92 (t, *J* = 7.3 Hz, 1H, aromatic), 3.57 (dd, *J* = 6.2, 4.1 Hz, 4H, $2CH_2$), 3.37 (dd, *J* = 6.2, 4.1 Hz, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.29, 154.15, 151.64, 150.87, 150.20, 150.12, 137.32, 136.09, 130.95, 130.61, 129.26, 126.84, 122.73, 120.35, 116.33, 113.58, 49.04, 47.26; ESI-MS: *m/z* $[M+H]^+$ 404; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{23}N_3OCl$ = 404.1530, found = 404.1533.

(*E*)-1-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(2-chloropyridin-3-yl)prop-2-en-1-one, 3f: Pale yellow solid. m.p. 146-148°C. FT-IR (KBr): 2923, 2844, 1658, 1595, 1395, 1303, 1189, 1111, 1012 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.40 (dd, *J* = 4.7, 1.7 Hz, 1H, aromatic), 8.03 (d, *J* = 2.1 Hz, 1H, aromatic), 8.02-7.99 (m, 1H, aromatic), 7.97 (d, *J* = 9.0 Hz, 2H, aromatic), 7.52 (d, *J* = 15.7 Hz, 1H, CH), 7.35 (s, 2H, aromatic), 7.34 (d, *J* = 3.0 Hz, 2H, aromatic), 7.33-7.30 (m, 1H, aromatic), 7.30-7.27 (m, 1H, aromatic), 6.90 (d, *J* = 9.0 Hz, 2H, aromatic), 3.58 (s, 2H, CH_2), 3.42-3.39 (m, 4H, $2CH_2$), 2.62-2.59 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.00, 154.25, 151.51, 149.99, 149.19, 137.56, 136.99, 136.01, 130.82, 129.09, 128.26, 127.20, 126.73, 122.68, 113.26, 62.85, 52.55,

47.02; ESI-MS: *m/z* $[M+H]^+$ 418; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{25}H_{25}N_3OCl$ = 418.1686, found = 418.1687.

(*E*)-3-(2-Chloropyridin-3-yl)-1-(4-morpholinophenyl)prop-2-en-1-one, 3g: Yellow solid. m.p. 151-153°C. FT-IR (KBr): 2847, 1651, 1591, 1390, 1305, 1184, 1116, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.41 (dd, *J* = 4.7, 1.8 Hz, 1H, aromatic), 8.05 (d, *J* = 1.8 Hz, 1H, aromatic), 8.04-7.98 (m, 3H, aromatic), 7.52 (d, *J* = 15.7 Hz, 1H, CH), 7.32 (dd, *J* = 7.9, 4.8 Hz, 1H, aromatic), 6.92 (d, *J* = 9.0 Hz, 2H, aromatic), 3.89-3.85 (m, 4H, $2CH_2$), 3.38-3.33 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.24, 154.33, 151.54, 150.08, 137.29, 136.06, 130.82, 130.48, 127.91, 126.68, 122.72, 113.26, 66.46, 47.27; ESI-MASS: *m/z* $[M+H]^+$ 329; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{18}H_{18}N_2O_2Cl$ =329.1057, found = 329.1062.

(*E*)-3-(2-Chloropyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one, 3h: Pale yellow solid. m.p. 110-112°C. FT-IR (KBr): 2911, 1650, 1590, 1394, 1303, 1183 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.41 (d, *J* = 2.4 Hz, 1H, aromatic), 8.04-7.97 (m, 4H, aromatic), 7.53-7.49 (d, *J* = 6.9 Hz, 1H, aromatic), 7.33-7.30 (m, 1H, aromatic), 6.88-6.86 (d, *J* = 9.1 Hz, 2H), 3.84 (m, 4H, $2CH_2$), 2.72 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 186.99, 152.98, 151.52, 150.04, 137.14, 136.07, 131.14, 130.52, 127.03, 126.71, 122.72, 113.61, 50.22, 25.79; ESI-MASS: *m/z* $[M+H]^+$ 345.

(*E*)-3-(2-Chloro-5-phenylpyridin-3-yl)-1-(4-(4-phenylpiperazin-1-yl)phenyl)prop-2-en-1-one, 3i: Pale yellow solid. m.p. 215-217°C. FT-IR (KBr): 2834, 1590, 1381, 1286, 1222, 1182 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.60 (d, *J* = 2.4 Hz, 1H, aromatic), 8.17 (d, *J* = 2.4 Hz, 1H, aromatic), 8.07 (d, *J* = 15.7 Hz, 1H, CH), 8.03 (d, *J* = 9.0 Hz, 2H, aromatic), 7.62 (d, *J* = 1.5 Hz, 1H, aromatic), 7.60 (s, 1H, aromatic), 7.58 (s, 1H, aromatic), 7.53 (dd, *J* = 8.2, 6.7 Hz, 2H, aromatic), 7.49-7.46 (m, 1H, aromatic), 7.31 (dd, *J* = 7.7, 6.4 Hz, 2H, aromatic), 6.99-6.96 (m, 4H, aromatic), 6.92 (d, *J* = 7.3 Hz, 1H, aromatic), 3.59-3.55 (m, 4H, $2CH_2$), 3.38-3.35 (m, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.27, 154.21, 150.91, 150.34, 148.38, 157.37, 136.35, 136.16, 134.49, 131.03, 130.33, 129.35, 129.30, 128.80, 127.72, 127.20, 127.03, 120.38, 116.37, 113.60, 49.07, 47.28; ESI-MS: *m/z* $[M+H]^+$ 480; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{30}H_{27}N_3OCl$ = 480.1843, found = 480.1844.

(*E*)-1-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(2-chloro-5-phenylpyridin-3-yl)prop-2-en-1-one, 3j: Pale yellow solid. m.p. 154-156°C. FT-IR (KBr): 2925, 1652, 1591, 1383, 1224, 1184, 1055, 1003 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.60 (d, *J* = 2.3 Hz, 1H, aromatic), 8.16 (d, *J* = 2.3 Hz, 1H, aromatic), 8.08 (dt, *J* = 15.7, 4.3 Hz, 2H, aromatic), 7.97 (dd, *J* = 16.7, 5.5 Hz, 3H aromatic), 7.74 (d, *J* = 8.9 Hz, 1H, aromatic), 7.62-7.59 (m, 2H aromatic), 7.51 (d, *J* = 8.2 Hz, 3H, aromatic), 7.36 (s, 2H, aromatic), 6.90 (d, *J* = 8.9 Hz, 2H aromatic), 6.71 (d, *J* = 8.7 Hz, 1H aromatic), 3.60 (s, 2H, CH_2), 3.44-3.41 (m, 4H, $2CH_2$), 2.63 (s, 4H, $2CH_2$); ^{13}C NMR (110 MHz, $CDCl_3$): δ 187.12, 154.29, 150.27, 148.27, 137.16, 136.11, 130.93, 129.28, 129.20, 128.72, 128.35, 127.35, 127.13, 113.37, 113.03, 62.89, 52.58, 47.04, 46.79, 29.68; ESI-MS: *m/z* $[M+H]^+$ 494; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{31}H_{29}N_3OCl$ = 494.1999, found = 494.2000.

(*E*)-3-(2-Chloro-5-phenylpyridin-3-yl)-1-(4-morpholinophenyl)prop-2-en-1-one, 3k: Pale yellow solid. m.p. 183-185°C. FT-IR (KBr): 2851, 1652, 1595, 1421, 1386, 1226, 1193, 1118, 1054 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.60 (d, *J* = 2.2 Hz, 1H, aromatic), 8.17 (d, *J* = 2.3 Hz, 1H, aromatic), 8.07 (d, *J* = 15.7 Hz, 1H, CH), 8.01 (d, *J* = 8.9 Hz, 2H, aromatic), 7.60 (dd, *J* = 11.3, 9.5 Hz, 3H, aromatic), 7.53 (t, *J* = 7.5 Hz, 2H, aromatic), 7.47 (t, *J* = 7.3 Hz, 1H, aromatic), 6.92 (d, *J* = 8.9 Hz, 2H, aromatic), 3.89-3.85 (m, 4H, $2CH_2$), 3.37-3.34 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.28, 154.40, 150.27, 148.32, 137.37, 136.29, 136.10, 134.42, 130.89, 130.24, 129.28, 128.73, 128.01, 127.13, 126.97, 113.32, 66.50, 47.36; ESI-MS: *m/z* $[M+H]^+$ 405; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{22}N_2O_2Cl$ = 405.1370, found = 405.1372.

(*E*)-3-(2-Chloro-5-phenylpyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one, 3l: Pale yellow solid. m.p. 155-157°C. FT-IR (KBr): 2912, 1651, 1594, 1393, 1290, 1187, 1025 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, *J* = 2.4 Hz, 1H, aromatic), 8.17 (d, *J* = 2.4 Hz, 1H, aromatic), 8.06 (d, *J* = 15.7 Hz, 1H, CH), 8.00 (d, *J* = 9.0 Hz, 2H, aromatic), 7.62-7.56 (m, 3H, aromatic), 7.53 (dd, *J* = 10.1, 4.7 Hz, 2H, aromatic), 7.48-7.45 (m, 1H, aromatic), 6.86 (d, *J* = 9.0 Hz, 2H, aromatic), 3.85-3.82 (m, 4H, $2CH_2$), 2.74-2.70 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.14, 153.13, 150.26, 148.33, 137.28, 136.36, 136.11, 134.53,

131.31, 130.34, 129.34, 128.81, 127.47 -126.73, 113.69, 50.31, 25.88; ESI-MS: *m/z* $[M+H]^+$ 421; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{22}N_2OSCl$ = 421.1141, found = 421.1145.

(*E*)-3-(2-Chloro-5-*p*-tolylpyridin-3-yl)-1-(4-(4-phenylpiperazin-1-yl)phenyl)prop-2-en-1-one, 3m: Pale yellow solid. m.p. 126-128°C. FT-IR (KBr): 3448 2824, 1650, 1596, 1498, 1385, 1226, 1190, 1030 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.59 (s, 1H, aromatic), 8.15 (s, 1H, aromatic), 8.02 (d, *J* = 8.3 Hz, 2H, aromatic), 7.58 (d, *J* = 15.8 Hz, 1H, CH), 7.50 (d, *J* = 7.2 Hz, 2H, aromatic), 7.32 (s, 4H, aromatic), 6.95 (dd, *J* = 20.2, 7.1 Hz, 6H, aromatic), 3.57 (s, 4H, $2CH_2$), 3.36 (s, 4H, $2CH_2$), 2.44 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.289, 154.14, 150.86, 149.86, 148.20, 138.82, 137.42, 136.21, 134.20, 133.17, 131.17, 130.17, 130.19, 130.00, 129.25, 127.70, 126.95, 120.32, 116.31, 116.56, 49.02, 47.24, 21.20; ESI-MS: *m/z* $[M+H]^+$ 494; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{31}H_{29}N_3OCl$ = 494.1999, found = 494.2000.

(*E*)-1-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(2-chloro-5-*p*-tolylpyridin-3-yl)prop-2-en-1-one, 3n: Pale yellow solid. m.p. 142-144°C. FT-IR (KBr): 2835, 1653, 1593, 1417, 1389, 1223, 1191, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.57 (d, *J* = 2.4 Hz, 1H, aromatic), 8.14 (d, *J* = 2.4 Hz, 1H, aromatic), 8.04 (t, *J* = 12.3 Hz, 1H, aromatic), 7.98 (d, *J* = 9.0 Hz, 2H, aromatic), 7.57 (d, *J* = 15.7 Hz, 1H, aromatic), 7.50 (d, *J* = 8.1 Hz, 2H aromatic), 7.37-7.34 (m, 4H, aromatic), 7.33-7.26 (m, 4H, aromatic), 6.90 (d, *J* = 9.1 Hz, 2H, aromatic), 3.58 (s, 2H, CH_2), 3.44-3.39 (m, 4H, $2CH_2$), 2.63-2.57 (m, 4H, $2CH_2$), 2.44 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.22, 154.36, 149.99, 148.19, 138.85, 137.53, 137.24, 134.22, 133.21, 130.17, 130.98, 130.27, 130.04, 129.24, 128.40, 127.37, 126.99, 113.42, 62.97, 52.66, 47.13, 21.25; ESI-MS: *m/z* $[M+H]^+$ 508; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{32}H_{31}N_3OCl$ = 508.2156, found = 508.2161.

(*E*)-3-(2-Chloro-5-p-tolylpyridin-3-yl)-1-(4-morpholinophenyl)prop-2-en-1-one, 3o: Pale yellow solid. m.p. 159-161°C. FT-IR (KBr): 2850, 1652, 1596, 1419, 1382, 1227, 1195, 1120 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, *J* = 2.2 Hz, 1H, aromatic), 8.15 (d, *J* = 2.1 Hz, 1H, aromatic), 8.06 (d, *J* = 15.8 Hz, 1H, CH), 8.01 (d, *J* = 8.9 Hz, 2H, aromatic), 7.58 (d, *J* = 15.7 Hz, 1H, CH), 7.50 (d, *J* = 8.0 Hz, 2H, aromatic), 7.33 (d, *J* = 7.8 Hz, 2H, aromatic), 6.92 (d, *J* = 8.9 Hz, 2H, aromatic), 3.90-3.85 (m, 4H, $2CH_2$), 3.38-3.33 (m, 4H, $2CH_2$), 2.43 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.18, 154.31, 149.86, 148.12, 138.76, 137.35, 136.12, 134.07, 113.03, 130.82, 130.05, 129.93, 127.90, 126.86, 126.71, 113.23, 66.24, 47.24; ESI-MS: *m/z* $[M+H]^+$ 419; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{25}H_{24}N_2O_2Cl$ = 419.1526, found = 419.1528.

(*E*)-3-(2-Chloro-5-*p*-tolylpyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one, 3p: Pale yellow solid. m.p. 103-105°C. FT-IR (KBr): 2920, 1653, 1591, 1393, 1287, 1181 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, *J* = 2.4 Hz, 1H, aromatic), 8.14 (d, *J* = 2.4 Hz, 1H, aromatic), 8.05 (d, *J* = 15.7 Hz, 1H, CH), 7.99 (d, *J* = 9.0 Hz, 2H, aromatic), 7.56 (t, *J* = 9.3 Hz, 1H, aromatic), 7.50 (d, *J* = 8.1 Hz, 2H, aromatic), 7.32 (d, *J* = 7.9 Hz, 2H, aromatic), 6.86 (d, *J* = 9.0 Hz, 2H, aromatic), 3.83 (dd, *J* = 6.2, 3.8 Hz, 4H, $2CH_2$), 2.73-2.70 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.17, 153.12, 149.98, 148.22, 138.88, 137.39, 136.26, 134.25, 133.20, 131.28, 130.24, 130.04, 127.02, 113.69, 50.31, 25.89, 21.25; ESI-MS: *m/z* $[M+H]^+$ 435; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{25}H_{24}N_2OSCl$ = 435.1298, found = 435.1301.

(*E*)-3-(2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)-1-(4-(4-phenylpiperazin-1-yl)phenyl)prop-2-en-1-one, 3q: Pale yellow solid. m.p. 168-170°C. FT-IR (KBr): 3447, 2826, 1650, 1597, 1385, 1227, 1163, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.56 (s, 1H, aromatic), 8.13 (s, 1H, aromatic), 8.04 (dd, *J* = 18.6, 12.3 Hz, 3H, CH), 7.63-7.53 (m, 3H, aromatic), 7.31 (t, *J* = 7.6 Hz, 2H, aromatic), 7.22 (t, *J* = 8.4 Hz, 2H, aromatic), 7.00-6.89 (m, 5H, aromatic), 3.57 (s, 4H, $2CH_2$), 3.36 (s, 4H, $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.18, 154.18, 150.85, 150.34, 148.12, 137.20, 135.36, 134.29, 132.27, 130.99, 130.36, 129.26, 128.96, 128.89, 127.63, 127.12, 120.35, 116.46, 116.32, 113.55, 49.03, 47.23; ESI-MS: *m/z* $[M+H]^+$ 498; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{30}H_{26}N_3OClF$ = 498.1749, found = 498.1749.

(*E*)-1-(4-(4-Benzylpiperazin-1-yl)phenyl)-3-(2-chloro-5-(4-fluorophenyl)pyridin-3-yl)prop-2-en-1-one, 3r: Pale yellow solid. m.p. 160-162°C. FT-IR (KBr): 2923, 1595, 1418, 1293, 1227, 1189 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.55 (d, *J* = 2.4 Hz, 1H, aromatic), 8.11 (d, *J* = 2.4 Hz, 1H, aromatic), 8.04 (t, *J* = 12.3 Hz, 1H, aromatic), 8.00 - 7.97 (m, 2H), 7.60 - 7.56 (m, 3H, aromatic), 7.36 - 7.33 (m,

6H, aromatic), 7.22 - 7.20 (m, 1H, aromatic), 6.90 (t, J = 6.0 Hz, 2H, aromatic), 3.59 (s, 2H CH_2), 3.43 - 3.40 (m, 4H $2CH_2$), 2.62 (s, 4H $2CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 187.11, 154.29, 150.32, 148.08, 137.07, 135.35, 134.28, 132.24, 130.95, 130.38, 129.28, 128.95, 128.39, 127.44, 127.29, 116.44, 116.27, 116.14, 113.41, 113.07, 62.82, 52.52, 46.99; ESI-MS: *m/z* $[M+H]^+$ 512.

(*E*)-3-(2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)-1-(4-morpholinophenyl)prop-2-en-1-one, 3s: Pale yellow solid. m.p. 192-194°C. FT-IR (KBr): 3019, 1655, 1599, 1420, 1381, 1221, 1120 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 2.4 Hz, 1H, aromatic), 8.12 (d, J = 2.4 Hz, 1H, aromatic), 8.06 (d, J = 15.8 Hz, 1H, CH), 8.03-7.99 (m, 2H, aromatic), 7.60-7.56 (m, 3H, aromatic), 7.22 (t, J = 8.6 Hz, 2H, aromatic), 6.92 (d, J = 9.0 Hz, 2H, aromatic), 3.89-3.86 (m, 4H, $2CH_2$), 3.38-3.33 (m, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.19, 164.43, 161.95, 154.39, 150.28, 148.10, 137.22, 135.32, 134.25, 130.89, 128.94, 128.85, 126.99, 116.44, 116.23, 113.28, 66.48, 47.29; ESI-MS: *m/z* $[M+H]^+$ 423; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{21}N_2O_2ClF$ = 423.1276, found = 423.1279.

(*E*)-3-(2-Chloro-5-(4-fluorophenyl)pyridin-3-yl)-1-(4-thiomorpholinophenyl)prop-2-en-1-one, 3t: Pale yellow solid. m.p. 115-117°C. FT-IR (KBr): 2915, 1648, 1591, 1515, 1415, 1290, 1224, 1176, 1090 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H, aromatic), 8.12 (s, 1H, aromatic), 8.05 (d, J = 15.7 Hz, 1H, CH), 7.99 (d, J = 8.5 Hz, 2H, aromatic), 7.58 (dd, J = 8.7, 5.9 Hz, 3H, aromatic), 7.22 (t, J = 8.4 Hz, 2H, aromatic), 6.86 (d, J = 8.6 Hz, 2H, aromatic), 3.85-3.82 (m, 4H, $2CH_2$), 2.72 (d, J = 4.2 Hz, 4H, $2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 187.03, 153.09, 150.33, 148.15, 137.15, 135.39, 134.39, 132.27, 131.32, 130.37, 129.04, 128.96, 127.15, 116.53, 116.32, 113.74, 38.85, 24.88; ESI-MS: *m/z* $[M+H]^+$ 439; HRMS (ESI): $[M+H]^+$ *m/z* calcd for $C_{24}H_{21}N_2OSClF$ = 439.1047, found = 439.1044.

Anti-bacterial activity

The anti-bacterial activities of prepared compounds were evaluated against two Gram-positive (*Bacillus megaterium*, *Staphylococcus aureus*) and two Gram-negative organisms (*Salmonella typhi and Escherichia coli*) by agar well plate method by using streptomycin as standard. The anti-fungal activities of prepared compounds were evaluated against yeast (*Candida albicans*) 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 inhibitions. 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 ºC 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 ºC to allow the compounds to diffuse into the agar, and then subsequently incubated for 24 h at 37 ºC for bacteria and 48 h at 28 ºC for fungi. Zone diameters were expressed in mm using calibrated scale. Experiment was triplicate to minimize the deviations.

Determination of MIC

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) studies against all above microbial strains. The concentrations of test samples were serially diluted from 500 to 1.9µg/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 ºC 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.

DPPH free radical scavenging assay

Assay for the scavenging of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was done. Briefly,

in a 96-well micro plate, 25 µL of test sample dissolved in DMSO (1 mg/mL), 100 µL of 0.1 M tris-HCl buffer (*p*H 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 for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as (1-B/ A) × 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)] cation was performed as described by Walker and Everette with suitable modifications[9]. Briefly, 100 mL stock solution of $ABTS^{\cdot+}$ (0.5 mM) was prepared by addition of 1 mL potassium persulfate (6.89 mM PBS, *p*H 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 compounds in 100 µL of methanol followed by 190 µL of $ABTS^{\cdot+}$ in a 96-well microplate. Absorbance of decolorized $ABTS^{\cdot+}$ was measured at 734 nm after 15 min incubation in the dark on a BioTeksynergy4 multi-mode microplate reader. For each test sample a separate blank sample (devoid of $ABTS^{+}$) was used for background subtraction. The percentage of $ABTS^{\cdot+}$ scavenging was calculated applying following formula:

$\%ABTS^{\cdot+}$ scavenging = [(Absorbance control– Absorbance test) / Absorbancecontrol×100]

Various serial dilutions of active compounds were prepared and tested for determination of SC_{50} values. Suitable regression analysis was applied for calculation of SC_{50}.

α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity was determined as per our earlier reported method[9]. 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, 10 µL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 µL of 100 mM-phosphate buffer (*p*H 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 after 15 minutes incubation at 405 nm spectrophotometrically (Spectra Maxplus 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) x 100 where [A] represents absorbance of control without test samples, and [B] represents absorbance in the presence of test samples.

Conclusion

In conclusion, target compounds chalcones **3a-t** has been prepared by the reaction of nicotinaldehydes **1a-e** with phenylethanones **2a-d** in the presence of base at RT. The compounds were evaluated for their anti-microbial, free-radical scavenging and α-glucosidase inhibitory activities. Compounds **3d** and **3h** have been identified as potent anti-fungal agents. Compounds **3c**, **3h**, **3k-m** and **3q** have shown α-glucosidase inhibitory activity.

Supplementary Information

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

Acknowledgements

B. China Raju acknowledges Science and Engineering Research Board (SERB), Department of Science and Technology (DST) New Delhi for the financial support (EEQ/2017/000314).

References

1 Chunlin Z, Wen Z, Chunquan S, Wannian Z, Chengguo X & Zhenyuan M, *Chem Rev*, 117 (2017) 7762.

2 Avila H P, Smania E F, Monache F D & Smania A, *Bioorg Med Chem*, 16 (2008) 9790.

3 (a) Sortino M, Delgado P, Juarez S, Quiroga J, Abonia R, Insuasty B, Nogueras M, Rodero L, Garibotto F M, Enriz R D & Zacchino S A, *Bioorg Med Chem*, 15 (2007) 484; (b) Vargas M L Y, Castelli M V, Kouznetsov V V, Urbino G J M, Lopez S N, Sortino M, Enriz R D, Ribas J C & Zacchino S, *Bioorg Med Chem*, 11 (2003) 1531; (c) Lopez S N, Castelli M V, Zacchino S A, Dominguez J N, Lobo G, Charris-Charris J, Cortes J C, Ribas J C, Devia C, Rodriguez A M & Enriz R D, *Bioorg Med Chem*, 8 (2001) 1999.

4 Cheng J H, Hung C F, Yang S C, Wang J P, Won S J & Lin C N, *Bioorg Med Chem*, 16 (2008) 7270.

5 (a) Katsori A M & Hadjipavlou-Litina D, *Curr Med Chem*, 16 (2009) 1062; (b) Achanta G, Modzelewska A, Feng L, Khan S R & Huang P, *Mol Pharmacol*, 70 (2006) 426;

(c) Modzelewska A, Pettit C, Achanta G, Davidson N E, Huang P & Khan S R, *Bioorg Med Chem*, 14 (2006) 3491; (d) Kumar S K, Hager E, Pettit C, Gurulingappa H, Davidson N E & Khan S R, *J Med Chem*, 46 (2003) 2813.

6 Hadjipavlou-Litina D, Magoulas G E, Krokidis M & Papaioannou D, *Eur J Med Chem*, 45 (2010) 298.

7 Ajay A, Singh V, Singh S, Pandey S, Gunjan S, Dubey D, Sinha S K, Singh B N, Chaturvedi V, Tripathi R, Ramachandran R & Tripathi R P, *Bioorg Med Chem*, 18 (2010) 8289.

8 Kumar R, Mohanakrishnan D, Sharma A, Kaushik N K, Kalia K, Sinha A K & Sahal D, *Eur J Med Chem*, 45 (2010) 5292.

9 Chimenti F, Fioravanti R, Bolasco A, Chimenti P, Secci D, Rossi F, Yanez M, Orallo F, Ortuso F & Alcaro S, *J Med Chem*, 52 (2009) 2818.

10 Srinivasan B, Johnson T E, Lad R & Xing C, *J Med Chem*, 52 (2009) 7228.

11 Hayat F, Moseley E, Salahuddin A, Van Zyl R L & Azam A, *Eur J Med Chem*, 46 (2011) 1897.

12 Gangadasu B, Raju B C & Rao V J, *J Heterocycl Chem*, 46 (2009) 1213.

13 Rao V J, Narender P, Srinivas U, Ravinder M, Rao B A, Ramesh Ch, Harakishore K, Gangadasu B & Murthy U S N, *Bioorg Med Chem*, 14 (2006) 4600.

14 Narender P, Ravinder M, Sadhua P S, Raju B C, Ramesh Ch & Rao V J, *Helv Chim Acta*, 92 (2009) 959.

15 Narender P, Srinivas U, Gangadasu B, Biswas S & Rao V J, *Bioorg Med Chem Lett*, 15 (2005) 5378.

16 Gangadasu B, Ram Reddy M J, Ravinder M, Kumar S B, Raju B C, Kumar K P, Murthy U S N & Rao V J, *Eur J Med Chem*, 44 (2009) 4661.

17 Raju B C & Suman P, *Chem Eur J*, 16 (2010) 11840.

18 Dayakar Ch, Mounika L, Rajkumar K, Zehra A, Murthy T R, Kalivendi S V, Tiwari A K & Raju B C, *Indian J Chem*, 57B (2018) 98.

19 Shivaraj B, Hariprasad K S, Rathod B B, Prakasham R S & Raju B C, *Indian J Chem*, 58B (2019) 497.

20 Dayakar Ch, Suman P, Rajkumar K, Murthy T R, Kalivendi S V & Raju B C, *Med Chem Res*, 27 (2018) 80.

21 Shivaraj B, Hariprasad K S, Rathod B B, Prakasham R S & Raju B C, *Indian J Chem*, 58B (2019) 497.

22 Gangadasu B, Narender P, Kumar S B, Ravinder M, Rao B A, Ramesh Ch, Raju B C & Rao V J, *Tetrahedron*, 62 (2006) 8398.

23 National Committee for Clinical Laboratory Standards (NCCLS), Nat. Comm. Clini. Lab. Standards Villanova (1982) 242.

24 Raju B C, Tiwari A K, Kumar J A, Ali A Z, Agawane S B, Saidachary G & Madhusudana K, *Bioorg Med Chem*, 18 (2010) 358.