Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 4 pp. 637–644, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND CHARACTERIZATION OF (E)-N'-(SUBSTITUTED BENZYLIDENE)ISONICOTINOHYDRAZIDE DERIVATIVES AS POTENT ANTIMICROBIAL AND HYDROGEN PEROXIDE SCAVENGING AGENTS

MANAV MALHOTRA[1,*], GAURAV SHARMA[2] and AAKASH DEEP[3]

[1]Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozepur Road, Moga-142001, India
[2]Department of Advanced Pediatric Centre Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India
[3]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001, India

Abstract: A series of (E)-N'-(substituted benzylidene)isonicotinohydrazide derivatives were synthesized by coupling isoniazid with differently substituted aldehydes and benzophenones in the presence of absolute ethanol along with catalytic amount of glacial acetic acid. The structure of all the synthesized compounds were confirmed and characterized by using various spectral technique like IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the synthesized compounds were evaluated for their antimicrobial activity in terms of zone of inhibition, minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration in camparison to the standard drugs. The results revealed that all synthesized compounds had shown potent to mild biological activity. Among the synthesized derivatives, (E)-N'-(3,4-dimethoxybenzylidene)isonicotinohydrazide **2e,** (E)-N'-(3,4,5-trimethoxybenzylidene)isonicotinohydrazide **2f** and (E)-N'-(4-hydroxy-3-methoxybenzylidene)isonicotinohydrazide **2g** were found to be the most effective antimicrobial compounds, whereas compounds **2g** and **2k** were the most potent antioxidants with significant hydrogen peroxide scavenging activity.

Keywords: hydrazones, lipophilicity, antibacterial, antifungal and antioxidant activity

Research and development of potent and effective antimicrobial agents represents one of the most important advances in therapeutics, not only in the control of serious infections, but also in the prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. Over the past decade, microbial infection becomes an important complication and a major cause of morbidity and mortality in immuno-compromised individuals such as those suffering from tuberculosis, cancer and AIDS and in organ transplantation cases (1). Antimicrobial agents are considered "miracle drugs" that are our leading weapons in the treatment of infectious diseases. Antimicrobial resistance is the ability of certain microorganism to withstand attack by antimicrobials, and the uncontrolled rise in resistant pathogens threatens lives and wastes limited healthcare resources. Life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world (2). Consequently, the development of newer antimicrobial agents will remain an important challenging task for medicinal chemists (3). So, there is an urgent need for identification of novel lead structures for the designing of new, potent and less toxic agents which ideally shorten the duration of therapy and are effective against resistant strains (4). Hydrazones belong to Schiff base family containing azomethine –NHN=CH protons and constitute an important class of compounds for new drug development (5). Day by day, the chemistry of carbon-nitrogen double bond of hydrazones is fast becoming the backbone of condensation reaction in benzo-fused N-heterocycles (6). Many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazones have been reported to possess, antimicrobial (7), antitubercular (8, 9) anticonvulsant (10), analgesic (11), anti-

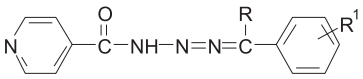
* Corresponding author: e-mail: manavmalhotra99@yahoo.in, phone: +91 9915172881

inflammatory (12, 13), antiplatelet (14), anticancer (15, 16), antifungal (17), antiviral (18), antitumoral (19, 20), antibacterial (21) and antimalarial (22) activities. Reaction of phenylhydrazine, the first hydrazine derivative was characterized by Fischer in 1875 (23). So, the antimicrobial resistance to a drug can be overcome by designing the new derivatives of hydrazones.

Among the important pharmacophores responsible for antimicrobial activity, the hydrazone scaffold is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial agents. Some widely used antibacterial drugs such as furacilin, furazolidone and ftivazide are known to contain hydrazone group.

Further, pharmacokinetic and cellular permeability of the drug can be increased by derivatization to bioreversible form of this drug, namely hydrazone. It is belived that hydrazone functional group increases the lipophilicity of parent amine and amides and results in enhancement of absorption through biomembranes and this enhanced lipophilicity of hydrazones, which enables them to cross bacterial and fungal membranes. Inspired by the above facts and in continuation of our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds (24–26) it was thought that it would be worthwhile to design and synthesize some new hydrazone derivatives and screen them for antimicrobial and antioxidant activities.

Table 1. Structures of compounds **2a**–**2k**.



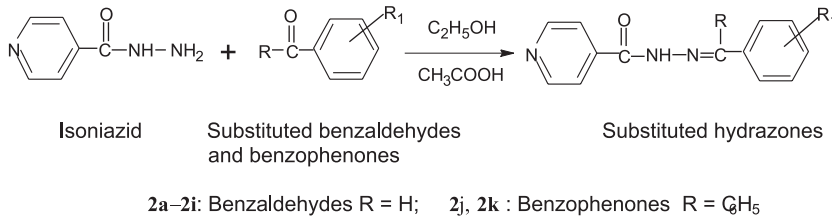
Compound	**R**	**R$_1$**
2a	H	H
2b	H	2-I
2c	H	3-I
2d	H	4-I
2e	H	3,4-OCH_3
2f	H	3,4,5-OCH_3
2g	H	3-OCH_3, 4-OH
2h	H	4-OC_3H_7
2i	H	4-$N(CH_3)_2$
2j	C_6H_5	–
2k	C_6H_5	4-Br

MATERIALS AND METHODS

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates silica gel 0.25 mm, 60 GF_{254}, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by iodine vapors/ultraviolet light as visualizing agents. The IR spectra were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^{1}H-NMR spectra were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C NMR spectra were recorded in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX aparatus.. Elemental analyses were performed on an ECS 4010 Elemental Combustion System (Costech). The necessary chemicals were purchased from Loba Chemie and Sigma Aldrich.

Synthesis of substituted aryl acid hydrazones derivatives 2a–2k

The synthetic strategies adopted to obtain the target compounds (**2a**–**2k**) are depicted in Scheme 1. The series of isonicotinohydrazide derivatives (**2a**–**2k**) was synthesized with good yields from commercially available materials. The type of substituted aldehydes and benzophenones are given in Table 1. The equimolar quantities of substituted benzaldehydes, or benzophenones (50 mmol) were refluxed with isonicotinic acid hydrazide (50 mmol)



Scheme 1. Synthesis of compounds **2a**–**k**

in absolute ethanol (50 mL) along with catalytic amount of glacial acetic acid. The reaction mixture was refluxed for 5–9 h and the completion of reaction was confirmed by TLC. After cooling and concentration of reaction mixture, the product was added to ice cold water. The precipitate was collected through filtration and dried in oven at low temperature. The crude products were recrystallized from absolute ethanol.

(E)-N'-(2-benzylidene)isonicotinohydrazide (2a)

Yield 82%, m.p. 205–208°C. IR (KBr, cm^{-1}): 3365, 3064, 1678, 1634, 1569. ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm): 12.25 (s, 1H, -NH-N=), 8.92 (d, 2H, pyridine, *J* = 4.5), 8.72 (s, 1H, N=C-H), 8.69 (d, 2H, pyridine, *J* = 3.9), 7.62–7.39 (m, 5H, benzylidene). ^{13}C-NMR (400 MHz, DMSO d_6, δ, ppm): 163.19, 149.75, 143.24, 139.76, 133.86, 130.78, 127.93, 121.72. MS (ESI) m/z = 284 (M+1). Analysis: calcd. for $C_{13}H_{11}N_3O$ (283.33): C 69.32, H 4.92, N 18.66%; found: C 69.45, H 4.95, N 18.53%.

(E)-N'-(2-Iodobenzylidene)isonicotinohydrazide (2b)

Yield 62%, m.p. 212–215°C. IR (KBr, cm^{-1}): 3239, 3018, 1671, 1659, 1542, 583. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.32 (s, 1H, -NH-N=), 8.79 (d, 2H, pyridine, *J* = 4.8 Hz), 8.69 (s, 1H, -N=C-H), 7.95 (d, 2H, pyridine, *J* = 4.3), 7.49 (d, 2H, benzylidene, *J* = 8.4), 7.15 (d, 2H, benzylidene, *J* = 7.9). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.14, 149.81, 143.15, 139.15, 137.19, 132.19, 130.11, 127.26, 122.37, 93.52. MS (ESI) m/z = 352 (M+1). Analysis: calcd. for $C_{13}H_{10}IN_3O_3$ (351.14): C 44.47, H 2.87, N 11.97%; found: C 44.53, H 2.85, N 11.93%.

(E)-N'-(3-Iodobenzylidene)isonicotinohydrazide (2c)

Yield 59%, m.p. 223–226°C. IR (KBr, cm^{-1}): 3219, 3018, 1669, 1641, 1559, 587. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.42 (s, 1H, -NH-N=), 8.75 (d, 2H, pyridine, *J* = 5.1 Hz), 8.65 (s, 1H, -N=C-H), 7.85 (d, 2H, pyridine, *J* = 4.5), 7.73 (s, 1H, benzylidene), 7.55 (t, 1H, benzylidene), 7.23 (d, 2H, benzylidene, *J* = 3.3 Hz). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.18, 149.28, 143.27, 139.22, 136.77, 135.15, 130.23, 128.19, 122.65, 94.17. MS (ESI) m/z = 352 (M+1). Analysis: calcd. for $C_{13}H_{10}IN_3O_3$ (351.14): C 44.47, H 2.87, N 11.97%; found: C 44.51, H 2.93, N 11.87%.

(E)-N'-(4-Iodobenzylidene)isonicotinohydrazide (2d)

Yield 69%, m.p. 242–245°C. IR (KBr, cm^{-1}): 3231, 3039, 1669, 1639, 1561, 569. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.10 (s, 1H, -NH-N=), 8.78 (d, 2H, pyridine, *J* = 4.8 Hz), 8.39 (s, 1H, -N=C-H), 7.84 (d, 2H, pyridine, *J* = 4.4), 7.79 (d, 2H, benzylidene, *J* = 8.7), 7.54 (d, 2H, benzylidene, *J* = 7.8). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.81, 149.74, 143.18, 139.15, 137.63, 132.18, 130.22, 122.29, 96.52. MS (ESI) m/z = 352 (M+1). Analysis: calcd. for $C_{13}H_{10}IN_3O_3$ (351.14): C 44.47, H 2.87, N 11.97%; found: C 44.45, H 2.95, N 11.91%.

(E)-N'-(3,4-dimethoxybenzylidene)isonicotinohydrazide (2e)

Yield 65%, m.p. 188–191°C. IR (KBr, cm^{-1}): 3232, 3047, 1675, 1645, 1560, 1249, 1026. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.82 (s, 1H, -NH-N=), 8.56 (d, 2H, pyridine, *J* = 4.8 Hz), 8.43 (s, 1H, -N=C-H), 7.80 (d, 2H, pyridine, *J* = 4.3), 7.35 (d, 2H, benzylidene, *J* = 8.4), 7.11 (s, 1H, benzylidene), 3.82 (s, 6H, 2CH3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.59, 152.98, 149.71, 149.12, 143.72, 139.82, 126.89, 122.82, 122.11, 114.28, 56.72. MS (ESI) m/z = 286 (M+1). Analysis: calcd. for $C_{15}H_{15}N_3O_3$ (285.32): C 63.15, H 5.30, N 14.73%; found: C 63.14, H 5.16, N 14.88%.

(E)-N'-(3,4,5-trimethoxybenzylidene)isonicotinohydrazide (2f)

Yield 66%, m.p. 209–212°C. IR (KBr, cm^{-1}): 3211, 3013, 1679, 1641, 1563, 1239, 1055. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.15 (s, 1H, -NH-N=), 8.85 (d, 2H, pyridine, *J* = 4.9 Hz), 8.75 (s, 1H, -N=C-H), 8.66 (d, 2H, pyridine, *J* = 4.3), 7.34 (s, 1H, benzylidene), 7.08 (s, 1H, benzylidene), 3.62 (s, 9H, $3CH_3$). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.17, 151.74, 149.72, 143.12, 141.27, 139.15, 127.45, 122.75, 107.19. MS (ESI) m/z = 316 (M+1). Analysis: calcd. for $C_{16}H_{17}N_3O_4$ (315.32): C 60.94, H 5.43, N 13.33%; found: C 60.89, H 5.45, N 13.36%.

(E)-N'-(4-hydroxy-3-methoxybenzylidene)isonicotinohydrazide (2g)

Yield 72%, m.p. 216–219°C. IR (KBr, cm^{-1}): 3336, 3256, 3029, 1661, 1639, 1542, 1292, 1029. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.86 (s, 1H, -NH-N=), 8.76 (d, 2H, pyridine, *J* = 4.3), 8.34 (s, 1H, -N=C-H), 7.80 (d, 2H, pyridine, *J* = 3.9), 7.32 (s, 1H, benzylidene), 7.09 (d, 2H, benzylidene, *J* = 8.2), 5.08 (s, 1H, OH, D_2O exchangeable), 3.82 (s, 3H, CH_3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.42, 151.94, 149.53, 147.65, 142.83, 140.38, 128.12, 123.74, 122.91, 116.85, 113.64, 55.89. MS (ESI) m/z = 272 (M+1). Analysis: calcd. for $C_{14}H_{13}N_3O_3$ (271.27): C 61.99, H 4.83, N 15.49%; found: C 61.97, H 4.76, N 15.58%.

Table 2. Antimicrobial screening results of the tested compounds.

Compound	Zone of inhibition (in mm)					
	Gram positive bacteria		Gram negative bacteria		Fungal strain	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
2a	15	19	14	18	13	11
2b	17	21	15	18	13	14
2c	19	20	17	19	13	14
2d	20	21	18	20	15	16
2e	24	30	22	29	15	15
2f	25	29	21	30	20	17
2g	24	29	20	31	20	19
2h	22	27	20	27	16	16
2i	21	23	19	24	15	15
2j	22	26	20	27	14	13
2k	19	21	16	20	15	14
Amoxicillin	25	30	21	30	–	–
Nystatin	–	–	–	–	20	19

Concentration for all compounds = 100 mg/mL.

(E)-N'-(4-Propoxybenzylidene)isonicotinohydrazide (2h)

Yield 58%, m.p. 155–158°C. IR (KBr, cm^{-1}): 3259, 3029, 1665, 1642, 1546, 1464, 1371, 1251, 1023. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.92 (s, 1H, -NH-N=), 8.77 (d, 2H, pyridine, *J* = 4.5 Hz), 8.39 (s, 1H, -N=C-H), 7.81 (d, 2H, pyridine, *J* = 4.1 Hz), 7.68 (d, 2H, benzylidene, *J* = 8.2 Hz), 7.01 (d, 2H, benzylidene, *J* = 7.5 Hz), 3.97 (t, 2H, CH_2), 1.75 (m, 2H, CH_2) 0.98 (t, 3H, CH_3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.49, 159.79, 149.82, 143.23, 139.79, 129.57, 125.55, 121.87, 114.18, 72.29, 22.36, 11.88. MS (ESI) m/z = 284 (M+1). Analysis: calcd. for $C_{16}H_{17}N_3O_2$ (283.33); C 67.83, H 6.05, N 14.83%; found: C 67.85, H 6.07, N 14.79%.

(E)-N'-(4-Dimethylaminobenzylidene)isonicotinohydrazide (2i)

Yield 69%, m.p. 207–210°C. IR (KBr, cm^{-1}): 3245, 3064, 1675, 1637, 1555, 1168. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.76 (s, 1H, -NH-N=), 8.75 (d, 2H, pyridine, *J* = 4.7), 8.30 (s, 1H, -N=C-H), 7.79 (d, 2H, pyridine, *J* = 4.2), 7.55 (d, 2H, benzylidene, *J* = 8.7), 6.75 (d, 2H, benzylidene, *J* = 8.2), 2.98 (s, 6H, CH_3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.18, 152.16, 149.77, 143.27, 139.86, 130.55, 121.79, 114.88, 41.56. MS (ESI) m/z = 269 (M+1). Analysis: calcd. for $C_{15}H_{16}N_4O$ (268.31): C 67.15, H 6.01, N 20.28%; found: C 67.18, H 5.95, N 20.31%.

(E)-N'-(Diphenylmethylene)isonicotinohydrazide (2j)

Yield 68%, m.p. 112–115°C. IR (KBr, cm^{-1}): 3244, 3062, 1691, 1662, 1569. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.52 (s, 1H, -NH-N=), 8.67 (d, 2H, pyridine, *J* = 4.6), 7.84 (d, 2H, pyridine, *J* = 4.1) 7.58 (m, 10 ArH, benzylidene). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.37, 156.18, 149.64, 139.79, 133.12, 131.28, 127.15, 122.84. MS (ESI) m/z = 302 (M+1). Analysis: calcd. for $C_{19}H_{15}N_3O$ (301.34): C 75.73, H 5.02, N 13.94%; found: C 75.82, H 5.05, N 13.82%.

(E)-N'-(4-Bromophenylphenylmethylene)isonicotinohydrazide (2k)

Yield 71%, m.p. 158–161°C. IR (KBr, cm^{-1}): 3256, 3054, 1689, 1662, 1557, 649. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.65 (s, 1H, -NH-N=), 8.68 (d, 2H, pyridine, *J* = 4.8), 7.72 (d, 2H, pyridine, *J* = 4.2), 7.50 (m, 4H, benzylidene), 7.37 (m, 5H, phenyl). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 163.33, 154.46, 149.77, 138.74, 131.81, 131.45, 131.15, 127.91, 125.43, 121.68. MS (ESI) m/z = 381 (M+1). Analysis: calcd. for $C_{19}H_{14}BrN_3O$ (380.24): C 60.02, H 3.71, N 11.05%; found: C 60.05, H 3.69, N 11.04%.

Pharmacology

Antibacterial studies

The newly synthesized compounds were screened for their antibacterial activity against some

multidrug resistant strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (27–29). The experiment was performed by disc diffusion method (30, 31). A standard inoculum (1–2 × 10^7 c.f.u./mL 0.5 McFarland standards) was introduced on the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile discs previously soaked with the test compound solution in DMSO of specific concentration 100 µg/disc were carefully placed on the agar culture plates. The plates were inverted and incubated for 24 h at 37°C. Amoxicillin was used as a standard drug. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition are given in Table 2. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially twofold diluted amount of the test compound and control were inoculated with approximately 5 × 10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

To obtain the minimum bactericidal concentration (MBC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 18–24 h of incubation at 35°C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The MIC and MBC values are given in Table 3.

Antifungal studies

The newly synthesized compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* in DMSO by agar diffusion method (32, 33). Sabourauds agar medium was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawing. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar medium was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37°C for 3–4 days. The fungal activity of each compound was compared with nystatin as a standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.

The nutrient broth, which contained logarithmic serially twofold diluted amount of test compound and controls was inoculated with approximately 1.6 × 10^4 – 6 × 10^4 c.f.u./mL. The cultures were incubated for 48 h at 35°C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as MIC. To obtain the minimum fungicid-

Table 3. MIC, MBC and MFC values of the tested compounds.

	B. subtilis		*S. aureus*		*P. aeruginosa*		*E. coli*		*C. albicans*		*A. niger*	
Compound	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
2a	6.25	12.5	12.5	50	50	100	3.12	6.25	12.5	25	25	50
2b	12.5	50	50	100	25	50	12.5	50	12.5	25	12.5	25
2c	3.12	6.25	12.5	25	6.25	12.5	25	50	25	50	12.5	25
2d	12.5	25	6.25	12.5	3.12	6.25	6.25	12.5	6.25	12.5	6.25	12.5
2e	3.12	6.25	1.56	1.56	1.56	3.12	3.12	6.25	3.25	6.25	3.12	6.25
2f	1.56	3.12	3.12	3.12	3.12	6.25	1.56	3.12	3.12	6.25	6.25	12.5
2g	3.12	6.25	6.25	12.5	1.56	3.12	3.12	6.25	1.56	3.12	1.56	3.12
2h	6.25	12.5	12.5	25	25	50	12.5	50	6.25	12.5	12.5	25
2i	12.5	25	6.25	12.5	3.12	6.25	6.25	12.5	6.25	12.5	6.25	12.5
2j	25	50	12.5	25	12.5	25	6.25	12.5	12.5	25	6.25	12.5
2k	3.12	6.25	12.5	50	12.5	50	25	50	6.25	12.5	12.5	25
Amoxicillin	1.56	3.12	1.56	3.12	1.56	3.12	1.56	3.12	–	–	–	–
Nystatin	–	–	–	–	–	–	–	–	1.56	3.12	1.56	3.12

Table 4. Hydrogen peroxide scavenging activity of synthesized compounds.

Compound	Scavenging of hydrogen peroxide at different concentrations (%)		
	100 µg	300 µg	500 µg
2a	41.52	39.68	39.68
2b	40.18	39.77	39.52
2c	39.57	41.65	41.92
2d	41.52	48.19	50.44
2e	42.88	38.75	39.26
2f	45.65	46.19	45.91
2g	51.18	54.75	54.33
2h	42.98	39.72	39.57
2i	46.85	44.32	43.87
2j	41.88	45.19	48.11
2k	49.32	53.19	52.33
BHA	65.51	67.15	69.39
Ascorbic acid	54.37	55.49	57.33

BHA = butylated hydroxyanisole

al concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 48 h of incubation at 35°C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculum was killed. The MIC and MFC values are given in Table 3.

Antioxidant activity

Antioxidant activity is determined in terms of hydrogen peroxide scavenging activity. The solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100, 300, and 500 µg/mL) of all the synthesized compounds were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of the synthesized compounds and the standard compounds were calculated using the following formula: Percentage scavenging $[H_2O_2] = [(A_0 - A_1)/ A_0] \times 100$, where A_0 was the absorbance of the blank, and A_1 was the absorbance in the presence of the sample and standards [34]. The percentage scavenging of hydrogen peroxide by the synthesized compounds at 100, 300 and 500 µg/mL concentrations were absorbed and results are summarized in Table 4.

RESULTS AND DISCUSSION

All the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *B. subtilis, S. aureus, P. aeruginosa* and *E. coli*. They were also evaluated for their *in vitro* antifungal potential against *C. albicans* and *A. niger.* Agar-diffusion method was used to determination of preliminary antibacterial activity. The results were recorded for each tested compound as the ability to create inhibitory zones of bacterial growth around the disc in mm. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **2e** (3,4-dimethoxyphenyl)**, 2f** (3,4,5-trimethoxyphenyl) and **2g** (4-hydroxy-3-methoxyphenyl) displayed excellent activity against Gram-positive bacteria *B. subtilis* and *S. aureus* and good activity against Gram-negative bacteria *P. aeruginosa* and *E. coli*. Compounds **2h** and **2j** showed moderate antibacterial activity, whereas compounds **2b** (2-iodophenyl), **2c** (3-iodophenyl) and **2d** (3-iodophenyl) are less active against all bacterial strains. Among all the synthesized derivatives, compound **2a** (phenyl) was found to be the least active compound against most of bacterial strains. The MIC measurement was determined for compounds that showed significant growth inhibition

zones. In this view, compound (**2f)** was equipotent to standard compound (MIC 1.56 µg/mL) against *B. subtilis* and *E. coli*. With regard to the activity against *S. aureus*, the best activity was displayed by compound (**2e**) (MIC 1.56 µg/mL), which represented potency similar to standard drug. The MBC of compounds **2e** and **2f** was found to be the same as MIC, but in majority of the compounds MBC was one or twofold higher than their corresponding MIC values.

Concerning the antifungal activity of the tested compounds, only two fungal strains have been selected *C. albicans* and *A. niger* and the results of antifungal screening data revealed that all the synthesized compounds showed variable degree of inhibition against the tested fungi. Agar diffusion method was used for determination of preliminary antifungal activity. The results were recorded for each tested compound as the ability to create inhibitory zones of fungal growth around the disc in mm. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good fungal inhibition as compared to standard drug – nystatin. Among the screened compounds, **2f** (3,4,5-trimethoxyphenyl) and **2g** (4-hydroxy-3-methoxyphenyl) exhibit the highest antifungal activity against both fungal strains. Among all the synthesized derivatives, compound **2a** (phenyl) was found to be the least active compound against both fungal strains. The results of antimicrobial activity data revealed that substitution at 3, 4 and 5 position of phenyl ring with electron releasing group impart good activity of the compounds. Further, the introduction of iodo group at 2, 3 and 4 position of phenyl ring ring do not impart any antimicrobial activity. It is intresting to note that the incorporation of methoxyphenyl group to hydrazone (**2e, 2f** and **2g)** impart antimicrobial activity of the synthesized compounds. Thus it has been noticed that the nature and position of the substituent has marked effect on antimicrobial activity of the synthesized compounds. The MIC measurement was determined for compounds that showed significant growth inhibition zones. It was found that compound **2g** was equipotent to standard compound (MIC 1.56 µg/mL) against *C. albicans* and *A. niger.* MFC of most of the compounds was found to twofold higher than their corresponding MIC results.

The evaluation of antioxidant activities was carried out by the method of scavenging of hydrogen peroxide. All the synthesized compounds exhibited potent hydrogen peroxide scavenging activities. From all the synthesized compounds, analogue **2g** was the most active with hydrogen peroxide scavenging by 54.75 and 54.33% at 300 and 500 µg/mL concentrations, respectively, followed by compound **2k** with scavenging of hydrogen peroxide by 53.19 and 52.33% at the same concentrations, respectively.

So, the present study showed that the synthesized compounds can be used as template for future development through structure modification and derivatization to design more potent and selective agents, which would be active against resistant strains for the treatment of microbial infections and having potent antioxidant activity.

REFERENCES

1. Turan-Zitouni G., Kaplancikli Z.A., Yildiz M.T., Chevallet P., Kaya D.: Eur. J. Med. Chem. 40, 607 (2005).
2. Dolman S.J., Gosselin F., Shea P.D., Davies, I.W.: J. Org. Chem. 71, 9548 (2006).
3. Verma A., Saraf S.K.: Eur. J. Med. Chem. 43, 897 (2008).
4. Murphy S.T., Case H.L., Ellsworth E., Hagen S., Husband M., Jonnides T., Limberakis C. et al.: Bioorg. Med. Chem. Lett. 17, 2155 (2007).
5. Rollas S., Kucukguzel S.G.: Molecules 12, 1910 (2007).
6. Rashed N., El Massry A.M., El Ashry E.-S.H., Amer A., Zimmer H.: J. Heterocycl. Chem. 27, 691 (1990).
7. Rollas S., Gulerman N., Edeniz H.: Farmaco 57, 171 (2002).
8. Imramovsky A., Polanc S., Vinsova J., Kocevar M., Jampitek J., Reckova Z., Kaustova J.A.: Bioorg. Med. Chem. 15, 2551 (2007).
9. Janin Y.: Bioorg. Med. Chem., 15, 2479 (2007).
10. Dimmock J.R., Vasishtha S.C., Stables J.P.: Eur. J. Med. Chem. 35, 241 (2000).
11. Lima P.C., Lima L.M., Silva K.C., Leda P.H., Miranda A.L.P., Fraga C.A.M., Barreiro E.J.: Eur. J. Med. Chem. 35, 187 (2000).
12. Salgin-Goksen U., Gokham-Keleci N., Gostal O., Koysal Y., Kilici E., Isik S., Aktay G., Ozalp M.: Bioorg. Med. Chem. 12, 3149 (2004).
13. Kalsi R., Shrimali M., Bhalla T.N., Barthwal J.P.: Ind. J. Pharm. Sci. 41, 353 (2006).
14. Silva G.A., Costa L.M.M., Brito F.C.F., Miranda A.L.P., Barreiro E.J., Fraga C.A.M.: Bioorg. Med. Chem. 12, 3149 (2004).
15. Savini L., Chiasserini L., Travagli V., Pellerano C., Novellino E., Consentino S., Pisano M.B.: Eur. J. Med. Chem. 39, 113 (2004).

16. Bijev A.: Lett. Drug Des. Discov. 3, 506 (2006).
17. Loncle C., Brunel J.M., Vidal N., Dherbomez M., Letourneux Y.: Eur. J. Med. Chem. 39, 1067 (2004).
18. Abdel-Aal M.T., El-Sayed W.A., El-Ashry E.H.: Arch. Pharm. Chem. Life Sci. 339, 656 (2006).
19. El-Hawash S.A.M., Abdel Wahab A.E., El-Demellawy M.A.: Arch. Pharm. Chem. Life Sci. 339, 14 (2006).
20. Cocco M.T., Congiu C., Lilliu V., Onnis V.: Bioorg. Med. Chem. 14, 366 (2005).
21. Capilla J., Serena C., Javier F., Ortoneda T., Guarro J.: Antimicrob. Agents Chemother. 47, 3976 (2003).
22. Walcourt A., Loyevsky M., Lovejoy D.B., Gordeuk V.R., Richardson, D.R.: Int. J. Biochem. Cell Biol. 36, 401 (2004).
23. Fischer E.: Ber. Deutsch. Bot. Ges. 8, 589 (1875).
24. Madhukar A., Kannappan N., Deep A., Kumar P., Kumar M., Prabhakar V.: Int. J. Chem. Tech. Res. 1, 1376 (2009).
25. Jha K.K., Samad A., Kumar Y., Shaharyar M., Khosa R.L., Jain J., Kumar V., Singh P.: Eur. J. Med. Chem. 45, 4963 (2010).
26. Malhotra M., Sharma S., Deep A.: Med. Chem. Res. (in press).
27. Poole K.: Curr. Opin. Investig. Drugs 4, 128 (2003).
28. Ohki R., Murata M.: J Bacteriol. 179, 1423 (1997).
29. Muto C.A., Jernigan J.A., Ostrowsky B.E., Richet H.M., Jarvis W.R., Boyce J.M., Farr B.M.: Infect. Control Hosp. Epidemiol. 24, 362 (2003).
30. Cruickshank R., Duguid J.P., Marmion B.P., Swain R.H.A.: Medicinal Microbiology, 12th edn., vol. II, p. 196, Churchill Livingstone, Edinburgh, London, New York 1975.
31. Collins A.H.: Microbiological Methods, 2nd edn., Butterworth, London 1976.
32. Khan Z.K.: In-vitro and in-vivo screening techniques for bioactivity screening and evaluation, in Proc. Int. Workshop UNIDO-CDRI, p. 210, 1997.
33. Antifungal Agents: Past, Present and Future Prospects, Varma R.S. Ed., National Academy of Chemistry and Biology, Lucknow, India 1998.
34. Gulcin I., Alici A.H., Cesur M.: Chem. Pharm. Bull. 53, 281 (2005).

Received: 16. 04. 2011