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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MANNICH BASES AS POTENT ANTIFUNGAL AND HYDROGEN PEROXIDE SCAVENGING AGENTS

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Abstract: In the present study, (E)-2-{[-2-(2,4-Dinitrophenyl)hydrazono]methyl}phenol **(3)** was synthesized and used as key intermediate for the synthesis of new Mannich bases. All the synthesized compounds were evaluated for their antifungal activity against three fungal strains *Candida albicans*, *Candida tropicalis* and *Aspergillus niger* and antioxidant activity. The structure of these compounds was confirmed by IR, ^{1}H NMR and ^{13}C NMR studies. Most of the compounds exhibited moderate to significant activities.

Keywords: hydrazones, Mannich bases, antifungal and antioxidant activity

The emergence of multi-drug resistant strains of microorganisms is a problem of ever increasing significance. The therapeutic problem has achieved increasing importance in hospitalized patients, in immunosuppressed patients with AIDS or undergoing anticancer therapy and organ transplants. Consequently, the development of new antimicrobial agents will remain an important challenging task for medicinal chemists (1). So, there is an urgent need for identification of novel lead structure for the designing of new, potent and less toxic agents, which ideally shorten the duration of therapy and are effective against resistant strain (2). Hydrazones belong to Schiff base family containing azomethine –NHN=CH protons and constitute the important class of compounds for new drug development (3). Hydrazone have been reported to possess, antifungal (4), antioxidant (5), antimicrobial (6), antitubercular (7, 8), antileprotic (9), anticonvulsant (10), analgesic (11), anti-inflammatory (12), antiplatelet (13), anticancer (14, 15), antiviral (16), antitumor (17, 18) and antimalarial activity (19). Antifungal resistance to a drug can be counteracted by designed new derivatives (20). Further, pharmacokinetic and cellular permeability of drug can be modulated by derivatization to bioreversible forms of this drug, namely hydrazone and its Mannich bases (21). Preparation of Mannich bases of hydrazone enhanced lipid solubility. Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a secondary amine (22). It is believed that the Mannich base functional group can increase the lipophilicity of parent amines and amides, which results in the enhancement of absorption through bio-membranes (23). The lipophilicity of Mannich bases 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 (24–30), we report the synthesis of new Mannich bases and evaluated them for antifungal and antioxidant activity.

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

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thin layer chromatography (TLC). Silica gel plates (Kieselgel 0.25 mm, 60G F_{254}), 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 (ν, cm^{-1}) were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^{1}H-NMR spectra (δ, ppm) 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. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Loba Chemie and Sigma-Aldrich.

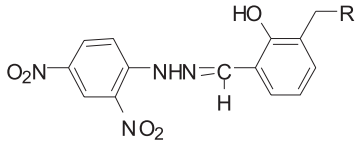
Chemistry

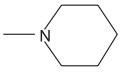
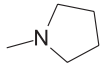
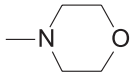
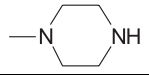
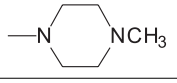
The synthetic pathway for the formation of target compounds is depicted in Scheme 1. Compounds **4a**–**4j** were readily prepared in good yields and purity. Equimolar quantity of 2,4-dinitrophenylhydrazine (**1**) and 2-hydroxybenzaldehyde **(2)** in 25 mL of absolute ethanol was refluxed for 5 h to form (E)-2-{[-2-(2,4-dinitrophenyl)hydrazono]methyl}phenol **(3).** The completion of reaction was confirmed by TLC. Further, compound **3** along with formaldehyde and substituted secondary amine was refluxed for 33–38 h in the presence of 50 mL of absolute ethanol and the pH was adjusted to 4 with hydrochloric acid to form titled compounds **(4a–4j).** The types of substituted secondary amines are specified in Table 1. The synthesized novel Mannich bases were characterized on the basis of the spectral and analytical studies.

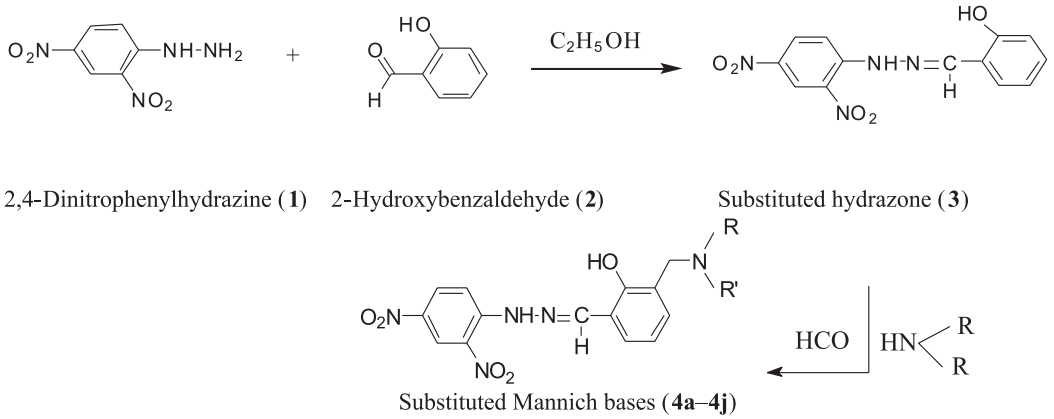
Synthesis of (E)-2-{[-2-(2,4-dinitrophenyl)hydrazono]methyl}phenol (3)

Equimolar quantity of 2,4-dinitrophenylhydrazine (1.98 g, 0.01 mol) and 2-hydroxybenzaldehyde (1.12 g, 0.01 mol) in 25 mL of absolute ethanol was refluxed for 5 h. The completion of reaction was confirmed by TLC. The reaction mixture was then

Table 1. Physical data of synthesized Mannich bases.



Compound	R	Molecular formula	Yield (%)	M.p. (°C)
4a	$-N(CH_3)_2$	$C_{16}H_{17}N_5O_5$	45	242–245
4b	$-N(C_2H_5)_2$	$C_{18}H_{21}N_5O_5$	61	215–218
4c	$-N(C_3H_7)_2$	$C_{20}H_{25}N_5O_5$	59	205–208
4d	$-N(C_4H_9)_2$	$C_{22}H_{29}N_5O_5$	53	212–215
4e	$-N(C_6H_5)_2$	$C_{26}H_{21}N_5O_5$	65	195–198
4f		$C_{19}H_{21}N_5O_5$	69	185–188
4g		$C_{18}H_{19}N_5O_5$	63	202–205
4h		$C_{18}H_{19}N_5O_6$	58	235–238
4i		$C_{18}H_{20}N_6O_5$	52	215–218
4j		$C_{19}H_{22}N_6O_5$	55	217–220



2,4-Dinitrophenylhydrazine (**1**) 2-Hydroxybenzaldehyde (**2**)

Scheme 1. Synthetic scheme for the formation of title compounds

poured in ice cold water and the precipitate obtained was filtered and dried in an oven at low temperature. The product was recrystallized from absolute ethanol.

IR (KBr; cm^{-1}): 3238, 3156, 2988, 2862, 2836, 1677, 1568, 1548, 1355, 1187. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.85 (s, 1H, phenyl), 8.48 (d, 2H, phenyl, *J* = 8.9 Hz), 8.11 (s, 1H, -N=C-H), 7.55 (d, 2H, phenol, *J* = 8.4 Hz), 7.38 (d, 2H, phenol, *J* = 7.9 Hz), 5.23 (s, 1H, OH, D_2O exchangable), 3.89 (s, 1H, -NH-N=). ^{13}C-NMR (400 MHz, DMSO d_6, δ, ppm): 160.82, 147.94, 143.17, 139.84, 134.52, 131.27, 129.56, 127.58, 120.18, 118.46. 118.05, 115.91. Analysis: calcd. for $C_{13}H_{10}N_4O_5$ (302.24): C 51.66, H 3.33, N 18.54%; found: C 51.72, H 3.35, N 18.46%.

Synthesis of substituted Mannich bases (4a–4j)

Compound **3** (728 mg, 0.0024 mol) along with (0.1 mL, 0.0036 mol) of formaldehyde and (0.0024 mol) of substituted secondary amine was placed in 100 mL round bottom flask to which 50 mL of absolute ethanol was added and the pH was adjusted to 4 with hydrochloric acid and refluxed for 33–38 h. The completion of reaction was confirmed by TLC. The reaction mixture was then poured to beaker and concentrated on water bath. It was allowed to cool at room temperature and then diethyl ether was added. The reaction mixture was kept for 3–5 h in refrigerator then was filtered and washed with *n*-hexane. The products were recrystallized from absolute ethanol.

(E)-2-[(Dimethylamino)methyl]-6-{[(2-(2,4-dinitrophenyl)hydrazono]methyl}phenol (4a)

IR (KBr; cm^{-1}): 3245, 3166, 2978, 2865, 2843, 1675, 1555, 1543, 1362, 1168. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.83 (s, 1H, phenyl), 8.41 (d, 2H, phenyl, *J* = 7.8 Hz), 8.09 (s, 1H, -N=C-H), 7.59 (d, 2H, phenol, *J* = 3.4 Hz), 6.54 (m, 1H, phenol), 5.23 (s, 1H, OH, D_2O exchangable) 3.92 (s, 1H, -NH-N=), 3.65 (s, 2H, Ar-CH_2-N), 2.25 (s, 6H, N-$(CH_3)_2$). ^{13}C-NMR (400 MHz, DMSO d_6, δ, ppm): 159.72, 147.18, 143.18, 138.19, 134.57, 132.61, 128.74, 127.19, 122.55, 119.89. 119.54, 118.37, 117.45, 55.18, 42.19. Analysis: calcd. for $C_{16}H_{17}N_5O_5$ (359.34): C 53.48, H 4.77, N 19.49%; found: C 53.43, H 4.79, N 19.52%.

(E)-2-[(Diethylamino)methyl]-6-{[2-(2,4-dinitrophenyl)hydrazono)]methyl}phenol (4b)

IR (KBr; cm^{-1}): 3259, 3174, 2982, 2857, 2845, 1664, 1549, 1537, 1354, 1188. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.74 (s, 1H, phenyl), 8.39 (d, 2H, phenyl, *J* = 8.2 Hz), 8.11 (s, 1H, -N=C-H), 7.35 (d, 2H, phenol, *J* = 3.2 Hz), 6.66 (m, 1H, phenol), 5.18 (s, 1H, OH, D_2O exchangable), 3.87 (s, 1H, -NH-N=), 3.61 (s, 2H, Ar-CH_2-N), 2.35 (m, 4H, N-$(CH_2)_2$), 1.15 (m, 6H, $2CH_3$). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.38, 147.35, 143.24, 139.12, 134.53, 132.68, 129.12, 128.39, 123.17, 121.41. 119.58, 118.26, 117.69, 52.13, 47.18, 15.92. Analysis: calcd. for $C_{18}H_{21}N_5O_5$ (387.39): C 55.81, H 5.46, N 18.08%; found: C 55.77, H 5.45, N 18.13%.

(E)-2-[(2-(2,4-dinitrophenyl)hydrazono]methyl-6-[(dipropylamino)methyl]phenol (4c)

IR (KBr; cm^{-1}): 3293, 3184, 2987, 2863, 2841, 1672, 1563, 1549, 1351, 1173. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.85 (s, 1H, phenyl), 8.34 (d, 2H, phenyl, *J* = 8.5 Hz), 8.15 (s, 1H, -N=C-H), 7.71 (d, 2H, phenol, *J* = 2.9 Hz), 6.81 (m, 1H, phenol), 5.55 (s, 1H, OH, D_2O exchangable), 3.82 (s, 1H, -NH-N=), 3.62 (s, 2H, Ar-CH_2-N), 2.31 (t, 4H, N-

$(CH_2)_2$), 1.51 (m, 4H, $2CH_2$), 1.13 (m, 6H, $2CH_3$). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.77, 147.58, 142.95, 138.55, 135.27, 132.34, 129.33, 127.71, 121.94, 119.22, 118.21, 117.52, 56.34, 52.19, 22.17, 13.58. Analysis: calcd. for $C_{20}H_{25}N_5O_5$ (415.44): C 57.82, H 6.07, N 16.86%; found: C 57.74, H 6.13, N 16.88%.

(E)-2-[(Dibutylamino)methyl]-6-{[2-(2,4-dinitrophenyl)hydrazono]methyl}phenol (4d)

IR (KBr; cm^{-1}): 3284, 3175, 2977, 2865, 2843, 1679, 1565, 1553, 1344, 1167. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.71 (s, 1H, phenyl), 8.35 (d, 2H, phenyl, *J* = 8.1 Hz), 8.07 (s, 1H, -N=C-H), 7.73 (d, 2H, phenol, *J* = 2.7 Hz), 6.85 (m, 1H, phenol), 5.47 (s, 1H, OH, D_2O exchangable), 3.88 (s, 1H, -NH-N=), 3.52 (s, 2H, Ar-CH_2-N), 2.31 (t, 4H, N-$(CH_2)_2$), 1.42 (m, 8H, $4CH_2$), 1.11 (t, 6H, $2CH_3$). ^{13}C-NMR (400 MHz, DMSO d_6, δ, ppm): 159.86, 147.18, 143.09, 139.15, 135.55, 132.91, 128.88, 127.61, 122.85, 121.93, 119.23, 118.24, 117.59, 56.31, 52.27, 30.92, 22.47, 13.77. Analysis: calcd. for $C_{22}H_{29}N_5O_5$ (443.50): C 59.58, H 6.59, N 15.79%; found: C 59.43, H 6.68, N 15.85%.

(E)-2-{[2-(2,4-dinitrophenyl)hydrazono]methyl}-6-6(diphenylamino)methyl]phenol (4e)

IR (KBr; cm^{-1}): 3281, 3177, 2974, 2862, 2845, 1671, 1583, 1549, 1356, 1171. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.69 (s, 1H, phenyl), 8.32 (d, 2H, phenyl, *J* = 7.7 Hz), 8.17 (s, 1H, -N=C-H), 7.84–6.95 (m, 13 ArH), 5.64 (s, 1H, OH, D_2O exchangable), 3.75 (s, 1H, -NH-N=), 3.52 (s, 2H, Ar-CH_2-N). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 158.75, 149.18, 147.21, 143.19, 139.15, 135.17, 131.18, 129.75, 129.93, 128.17, 122.16, 119.27, 118.35, 117.73, 49.13. Analysis: calcd. for $C_{26}H_{21}N_5O_5$ (483.48): C 64.59, H 4.38, N 14.49%; found: C 64.62, H 4.26, N 14.58%.

(E)-2-{[2-(2,4-dinitrophenyl)hydrazono]methyl}-6-(piperidin-1-ylmethyl)phenol (4f)

IR (KBr; cm^{-1}): 3285, 3169, 2963, 2861, 2842, 1666, 1574, 1552, 1348, 1154. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.72 (s, 1H, phenyl), 8.37 (d, 2H, phenyl, *J* = 7.3 Hz), 8.22 (s, 1H, -N=C-H), 7.69 (d, 2H, phenol, *J* = 2.8 Hz), 6.82 (m, 1H, phenol), 5.53 (s, 1H, OH, D_2O exchangable), 3.81 (s, 1H, -NH-N=), 3.59 (s, 2H, Ar-CH_2-N), 2.42 (t, 4H, N-$(CH_2)_2$), piperidine), 1.84 (m, 6H, $3CH_2$). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.24, 147.35, 143.23, 139.34, 135.19, 132.81, 129.43, 128.22, 123.26, 121.18, 119.37, 118.55, 117.51, 55.45, 52.19, 25.83. Analysis: calcd. for $C_{19}H_{21}N_5O_5$ (399.40): C 57.14, H 5.30, N 17.53%; found: C 57.11, H 5.35, N 17.51%.

(E)-2-{[2-(2,4-dinitrophenyl)hydrazono]methyl}-6-(pyrrolidin-1-ylmethyl)phenol (4g)

IR (KBr; cm^{-1}): 3286, 3175, 2965, 2858, 2843, 1663, 1572, 1554, 1345, 1169. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.77 (s, 1H, phenyl), 8.54 (d, 2H, phenyl, *J* = 7.5 Hz), 8.23 (s, 1H, -N=C-H), 7.65 (d, 2H, phenol, *J* = 2.7 Hz), 6.84 (m, 1H, phenol), 5.59 (s, 1H, OH, D_2O exchangable), 3.84 (s, 1H, -NH-N=), 3.84 (s, 2H, Ar-CH_2-N), 2.37 (t, 4H, N-$(CH_2)_2$, pyrrolidine), 1.52 (m, 4H, $2CH_2$, pyrrolidine). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.28, 147.26, 143.19, 139.35, 135.72, 132.59, 129.35, 128.18, 123.76, 122.19, 119.46, 118.66, 117.62, 57.15, 49.25, 25.18. Analysis: calcd. for $C_{18}H_{19}N_5O_5$ (385.37): C 56.10, H 4.97, N 18.17%; found: C 56.14, H 4.95, N 18.15%.

(E)-2-{[2-(2,4-dinitrophenyl)hydrazono]methyl}-6-(morpholinomethyl)phenol (4h)

IR (KBr; cm^{-1}): 3281, 3177, 2963, 2855, 2841, 1666, 1566, 1552, 1342, 1158. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.79 (s, 1H, phenyl), 8.47 (d, 2H, phenyl, *J* = 8.5 Hz), 8.55 (s, 1H, -N=C-H), 7.77 (d, 2H, phenol, *J* = 3.1 Hz), 6.91 (m, 1H, phenol), 5.52 (s, 1H, OH, D_2O exchangable), 3.82 (s, 1H, -NH-N=), 3.73 (s, 2H, Ar-CH_2-N), 3.42 (m, 4H, O-$(CH_2)_2$, morpholine), 2.31 (t, 4H, N-$(CH_2)_2$, morpholine). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.37, 147.25, 143.53, 139.54, 135.56, 132.55, 129.34, 128.51, 123.37, 122.19, 119.48, 118.54, 117.65, 66.52, 54.19, 52.54. Analysis: calcd. for $C_{18}H_{19}N_5O_6$ (401.37): C 53.86, H 4.77, N 17.45%; found: C 53.77, H 4.75, N 17.56%.

(E)-2-{[2-(2,4-dinitrophenyl)hydrazono]methyl}-6-(piperazin-1-ylmethyl)phenol (4i)

IR (KBr; cm^{-1}): 3284, 3173, 2958, 2847, 2845, 1673, 1564, 1553, 1345, 1168. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.75 (s, 1H, phenyl), 8.59 (d, 2H, phenyl, *J* = 8.8 Hz), 8.53 (s, 1H, -N=C-H), 7.79 (d, 2H, phenol, *J* = 2.9 Hz), 6.94 (m, 1H, phenol), 5.42 (s, 1H, OH, D_2O exchangable), 3.85 (s, 1H, -NH-N=), 3.71 (s, 2H, Ar-CH_2-N), 2.69 (m, 8H, $4CH_2$, piperazine). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.75, 147.78, 143.29, 139.51, 135.87, 132.66, 129.31, 128.47, 123.55, 112.21, 119.72, 118.72, 117.55, 54.18, 53.17, 46.72. Analysis: calcd. for $C_{18}H_{20}N_6O_5$ (400.39): C 54.00, H 5.03, N 20.99%; found: C 54.11, H 5.01, N 20.89%.

Table 2. *In Vitro* antifungal activity of the title compounds (**4a**–**4j**).

Compound	Minimum inhibitory concentration (µg/mL)		
	C. albicans (MTCC 8184)	*C. tropicalis* (MTCC 5158)	A. *niger* (MTCC 8189)
4a	25	12.5	12.5
4b	12.5	25	50
4c	25	50	25
4d	50	12.5	25
4e	3.12	6.25	3.12
4f	12.5	6.25	25
4g	12.5	12.5	25
4h	1.56	3.12	1.56
4i	3.12	1.56	6.25
4j	6.25	12.5	6.25
Clotrimazole (standard drug)	0.30	0.50	0.78

Table 3. Hydrogen peroxide scavenging activity of synthesized compounds

Compound	Scavenging of hydrogen peroxide at different concentration (%)		
	100 µg/mL	300 µg/mL	500 µg/mL
4a	41.52	39.68	39.68
4b	40.18	39.77	39.52
4c	38.72	41.15	40.72
4d	39.57	41.65	41.92
4e	41.52	48.19	50.44
4f	42.88	38.75	39.26
4g	45.82	43.32	43.87
4h	51.18	54.75	54.33
4i	49.32	53.19	52.33
4j	43.18	45.65	51.47
BHA	63.27	66.19	68.25
Ascorbic acid	51.47	53.45	55.38

(E)-2-((2-(2,4-dinitrophenyl)hydrazono)methyl)-6-((4-methylpiperazin-1-yl)methyl)phenol (4j)

IR (KBr; cm^{-1}): 3272, 3118, 2943, 2850, 2841, 1675, 1569, 1555, 1339, 1162. ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.72 (s, 1H, phenyl), 8.57 (d, 2H, phenyl, *J* = 8.9 Hz), 8.16 (s, 1H, -N=C-H), 7.72 (d, 2H, phenol, *J* = 2.9 Hz), 6.92 (m, 1H, phenol), 5.49 (s, 1H, OH, D_2O exchangable), 3.87 (s, 1H, -NH-N=), 3.63 (s, 2H, Ar-CH_2-N), 2.49 (m, 8H, 4CH_2, piperazine), 2.15 (s, 3H, CH_3). ^{13}C-NMR (400 MHz, DMSO-d_6, δ, ppm): 159.72, 147.54, 143.18, 139.59, 135.75, 132.53, 129.48, 128.43, 123.91, 112.28, 119.26, 118.24, 117.25, 54.72, 53.19, 49.72, 43.25. Analysis: calcd. for $C_{19}H_{22}N_6O_5$ (414.42): C 55.07, H 5.35, N 20.28%; found: C 55.13, H 5.33, N 20.24%.

Antifungal evaluation

Screening of finally synthesized compounds for their *in vitro* antifungal activity against fungal strain: *C. albicans* (MTCC 8184), *C. tropicalis* (MTCC 5158) and *A. niger* (MTCC 8189) was

assessed by serial twofold dilution technique. Clotrimazole was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 µg/mL. Twofold dilutions of test and standard compounds were prepared in Sabouraud dextrose broth I.P. Twofold dilutions of test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. fungi (31). The stock solution was serially diluted to give concentrations of 50–0.78 µg/mL in nutrient broth. The inoculum size was approximately 10^6 colony forming units (CFU/mL). The inoculum size was approximately 10^6 colony forming units (CFU/mL). The whole batch was incubated for 7 days for fungi at 35˚C for *A. niger* (MTCC 8189), 25˚C for *C. albicans* (MTCC 8184) and 28˚C for *C. tropicalis* (MTCC 8185). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compounds. The MIC for antifungal is given in Table 2.

Hydrogen peroxide scavenging activity

A 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 (32). The percentage scavenging of hydrogen peroxide by the synthesized compounds at 100, 300 and 500 µg/mL concentration were absorbed and results are summarized in Table 3.

RESULTS AND DISCUSSION

In this study novel Mannich bases have been synthesized and evaluated them for antifungal activity. The structures of all the newly synthesized compounds were confirmed by suitable spectroscopic methods such as IR, ^{1}H-NMR and ^{13}C-NMR. The IR spectra of all compound **4a**–**4j** showed absorption band at around 3293–3245, 3184–3118, 2987–2943, 2865–2841, 1679–1663, 1583–1549, 1555–1339 and 1188–1154 cm^{-1} regions, conforming the presence of OH, NH, CH, CH_2, C=N, C=O, C=C, NO_2, C-N, respectively. In the ^{1}H-NMR spectra, the signals of the respective prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of most compounds showed the characteristic phenyl proton δ 8.83–8.32 ppm, 1 H proton of -N=C-H at δ 8.59–8.07 ppm, 3 H protons of phenol were at around δ 7.84–6.54 ppm, 1 H proton of OH at δ 5.64–5.18 ppm, 1 H proton of –NH-N= at δ 3.92–3.75 ppm and 2 H protons of Ar-CH_2-N at δ 3.73–3.52 ppm. The ^{13}C-NMR spectra of most compounds have characteristic phenol signals appeared at δ 159.86–117.25, phenyl δ 149.18–118.21 ppm, -N=C-H δ 143.53–142.95 ppm, Ar-CH_2-N δ 57.15–49.13 ppm. The elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectral data of synthesized compounds were found in agreement with the assigned molecular structure. Of all the synthesized derivatives, compounds **4e, 4h,** and **4i** were the most active against the investigated strains as compared to the standards drugs. So, it was concluded that the presence of diphenylamine, morpholine and piperazine moiety, besides hydrazide functional group, was found to be essential for their high antifungal activity. It was also concluded from the results that antifungal activity increases with an increase in chain length from dimethylamine to dibutylamine. From all the synthesized derivatives, compounds **4h** with morpholine moiety was the most active with scavenging of hydrogen peroxide at 500 µg/mL concentration, followed by compound **4i** with piperazine moiety and **4e** having diphenyl moiety. The same correlation was found to be true in the case of antifungal activity, where the presence of similar substituents along with hydrazone led to an increase of biological activity as compared to the different substituents. So, the significant antifungal and antioxidant activity of compound may be due to the presence of diphenylamine, morpholine and piperazine moiety in addition to hydrazide functional group.

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