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Synthesis and Biological Evaluation of Some Novel Arylidene Hydrazides Derivatives

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Abstract

There has been considerable interest in the development of novel compounds with anticonvulsant, antioxidant, antibacterial, and antifungal activities. The present study explores the antimicrobial activity of some new arylidene hydrazide derivatives and correlates the effect on the antimicrobial potency by varying the substituents on hydrazide part of the arylidine hydrazide. Hydrazones possessing an azomethine–NHN=CH- proton constitute an important class of compounds for new drug developments; therefore, a series of arylidene hydrazides were synthesized with various aromatic aldehydes/ketones. The synthesis of title compounds was affected as outlined in the scheme. In this scheme O-chloro benzoic acid on reaction with aniline through Ullmann Reaction gave N-phenyl anthranilic acid. The esterification product of N-phenyl anthranilic acid followed by reaction with hydrazine hydrate yielded 2-phenyl amino benzoic acid hydrazide. These hydrazides were give arylidine hydrazide as title compounds. A total of 11 compounds were synthesized. The synthetic methods used are simple, rapid, and economical found to be accurate and reproducible. All the intermediates and title compounds were characterized by running TLC, determining M.P. and spectral studies such as IR and ^{1}H-NMR. The synthesized compounds showed mild-to-moderate antibacterial activity against Gram-positive *Bacillus subtilis* (MTCC441), *Bacillus cereus* (MTCC-7190) and Gram-negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus fumigates* (ATTC 9197). The bio-screening data revealed that 4b, 4f, 4g, 4h, and 4j moiety exhibited good antibacterial activity against all where 4a and 4c showed good antifungal activity. Among the compounds, 4a and 4c exhibited good antioxidant activity too.

Keywords: Antibacterial, Antifungal, Antioxidant, Arylidene hydrazide

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Introduction

Infectious diseases caused by bacteria and viruses have been increased dramatically in recent years. Despite many significant advances in antibacterial therapy, the widespread use and misuse of antibiotics have led to the emergence of bacterial resistance to antibiotics, which is a serious threat to the scientific community as well as public health. Arylidene hydrazones have received the attention of chemists due to their wide range of biological activities, which include anticonvulsant,[1-3] antifungal,[4-6] antibacterial,[7-9] anticancer,[10-12] antioxidant,[13,14] and antitubercular[15-17] activities. In the present study, it was envisaged that a drug molecule possessing the above-mentioned pharmacophore could be of advantage since it might possess anticonvulsant, antifungal, and antibacterial activities. An important objective of the study is to develop potent antifungal and antibacterial agents and anticonvulsant.

Materials and Methods

Infrared (IR) spectra were recorded on an Agilent Cary 630 Fourier transform infrared (FTIR) spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III HD 400 MHz spectrometer using TMS as an internal standard. DMSO was used as the solvents for dissolving the samples for NMR. All reactions were carried out using dry glassware. The chemicals used in this work were obtained from Sigma-Aldrich and Spectrochem Pvt. Ltd. and were used without further purification. Commercial grade solvents were used. Analytical thin-layer chromatography (T.L.C.) was performed on silica gel coated on aluminum sheets and was monitored using ultraviolet light of wavelength 254 nm. Column chromatography was performed on 60–120 mesh silica gel. Compounds were eluted by a mixture of hexane and ethyl acetate as required percentage.

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Chemistry

Synthesis of N-phenyl anthranilic acid ($C_{13}H_{11}NO_2$)

A mixture of o-chloro benzoic acid (1 mole), aniline (1 mole), copper power (0.2 g), and potassium carbonate (10 g) in 60 ml of iso-amyl alcohol were refluxed on a water bath for 8 h with occasional stirring. After the completion of the reaction, the mixture was allowed to cool at room temperature. The reaction mixture was filtered and acidified with conc. hydrochloric acid. The precipitate so obtained was again filtered and washed with hot water. The crude acid was dissolved in 0.1 N sodium hydroxide solution and reprecipitated by adding conc. hydrochloric acid. The crude acid was filtered and washed with water. The dried crude product (I) was recrystallized with alcohol. The completion of the reaction was monitored by T.L.C.[18,19]

Mobile phase: n-hexane: ethyl acetate (6:4), melting point: 189°C, yield: 4.2 g (42%).

Step: 2

Synthesis of methyl 2-(phenyl amino) benzoate ($C_{14}H_{13}NO_2$)

A solution of N-phenyl anthranilic acid (1 mole) in acetone was refluxed with dimethyl sulfate (2 mole) and anhydrous potassium carbonate (0.02 mole) on a water bath for 90 min. After the completion of the reaction, the reaction mixture was allowed to cool at room temperature and inorganic salt was filtered off. The filtrate was concentrated and after cooling to room temperature, poured into crushed ice. The precipitate so formed was filtered and washed with water, dried, and recrystallized (II) with methanol. The completion of the reaction was monitored by T.L.C.[20]

Melting point: 50°C, yield: 8.5 g (85%).

Step: 3

Synthesis of 2-phenyl amino benzoic acid hydrazide ($C_{13}H_{13}N_3O$)

A solution of methyl 2-(phenyl amino) benzoate (3 mole) was dissolved in ethanol and refluxed with 99% hydrazine hydrate (8 mole) on a water bath for 5–6 h. After the completion of the reaction, the reaction mixture was allowed to cool at room temperature and poured into a beaker containing crushed ice. The reaction mixture was allowed to stand for 1 h. The precipitate so formed was filtered and washed with water. The crude product was dried and recrystallized with ether. The completion of the reaction was monitored by T.L.C. and melting point: 116°C, yield: 6.5 g (92.8%).[9]

Step: 4

General synthesis of allylidene hydrazide

The reaction between 2-(phenylamino) benzohydrazide and different aldehydes (unimolar quantity) was done for 1 h at room temperature. The progress of the reaction was checked by performing T.L.C. in the solvent mixture of petroleum ether and ethyl acetate in the ration of 7:3. The target compounds were purified by column chromatography at different solvent mixture of petroleum ether and ethyl acetate in the ration of 7:3. The resulted compounds (**IV a-k**) were characterized by IR, ^{1}H-NMR, mass, and melting point.[21]

4a: Synthesis of 2-phenylamino benzoic acid (3-phenylallylidene) hydrazide

Compound IV a

Molecular formula: $C_{22}H_{19}N_3O$, Yield (85%), bluish. M.P.–180°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3457.29 (N-H elongation), 3051.32 (Ar. C-H elongation), 1670.33 (C=O elongation), 1638.41 (Ar.C=C elongation), 1528.25 (C=N elongation), 1317.71 (C-N elongation), 1030.59 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.32 (1H, s) 8.64 (1H, s), 8.48 (2H, d) 8.37 (1H, d), 7.99 (2H, d), 7.70 (2H, d), 7.60, 2H), 7.39 (2H, d), 7.36 (2H, d), 6.98 (1H, s), 6.88 (1H, s), 6.10 (1H, s), 5.60 (1H, s), 4.56 (1H, s). ESI MS of $C_{22}H_{19}N_3O$ found is 341.41.

Compound IV b

2-phenylamino benzoic acid (benzylidene) hydrazide: Molecular formula: $C_{20}H_{17}N_3O$, Yield (85%), brown. M.P.–160°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3446.59 (N-H elongation), 3049.26 (Ar. C-H elongation), 1669.21 (C=O elongation), 1636.42 (Ar.C=C elongation), 1526.92 (C=N elongation), 1318.29 (C-N elongation), 1028.28 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.12 (1H, s) 8.74 (1H, s), 8.56 (2H, d) 8.28 (2H, d), 8.19 (1H, d), 7.71 (2H, d), 7.69 (2H, d), 7.60 (2H, d), 7.39 (2H, d), 7.32 (2H, d), 6.99 (1H, s), 6.70(1H, s), 6.0 (1H, s), 4.56 (1H, s). ESI MS of $C_{20}H_{17}N_3O$ found is 315.37.

Compound IV c

2-phenylaminobenzoic acid (2-hydroxybenzylidene) hydrazide: Molecular formula: $C_{20}H_{17}N_3O_2$, yield (85%), brown. M.P.–160°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3455.46 (N-H elongation), 3290.42 (Ar. O-H elongation), 3047.44 (Ar.C-H elongation), 1667.49 (C=O elongation), 1635.67 (Ar.C=C elongation), 1525.29 (C=N elongation), 1316.48 (C-N elongation), 1027.36 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.12 (1H, s), 8.74 (1H, s), 8.56 (2H, d), 8.28 (2H, d), 8.19 (1H, d), 7.71 (2H, d), 7.69 (2H, d), 7.60 (2H, d), 7.39 (2H, d), 7.32 (2H, d), 6.99 (1H, s), 6.70 (1H, s), 6.0 (1H, s), 4.56 (1H, s). ESI MS of $C_{20}H_{17}N_3O_2$ found is 315.37.

Compound IV d

2-phenylaminobenzoic acid (4-hydroxybenzylidene) hydrazide: Molecular formula: $C_{20}H_{17}N_3O_2$, yield (91%), dark yellow. M.P.–216°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3454.39 (N-H elongation), 3289.52 (Ar. O-H elongation), 3046.56 (Ar.C-H elongation), 1666.36 (C=O elongation), 1634.66 (Ar.C=C elongation), 1524.24 (C=N elongation), 1315.66 (C-N elongation), 1026.36 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.28 (1H, s), 9.82 (1H, s), 8.92 (1H, s) 8.28 (2H, d), 8.22 (2H, d), 8.19 (2H, d), 7.74 (2H, d), 7.60 (2H, d), 7.59 (2H, d), 6.50 (1H, s), 4.51 (1H, s). ESI MS of $C_{20}H_{17}N_3O_2$ found is 315.37.

Compound IV e

2-phenylaminobenzoic acid (4-chlorobenzylidene) hydrazide: Molecular formula: $C_{20}H_{16}$ ClN_3O, $C_{20}H_{16}$ ClN_3O, yield (86%), black. M.P.–180°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3453.78 (N-H elongation), 3044.74 (Ar. C-H elongation), 1663.43 (C=O elongation), 1633.36 (Ar.C=C elongation), 1523.98 (C=N elongation), 1323.65 (C-N elongation), 1025.52 (Ar. C-C elongation), 712.59 (Ar. C-Cl elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ (in ppm): 11.22 (1H, s) 8.98 (1H, s), 8.32 (2H, d) 8.22 (2H, d), 8.19 (1H, d), 7.78 (2H, d), 7.59 (2H, d), 6.83 (2H, d), 6.83 (2H, d), 6.83 (2H, d), 6.38 (1H, s), 4.63 (1H, s). ESI MS of $C_{20}H_{16}ClN_3O$ found is 349.81.

Compound IV f

2-phenylaminobenzoic acid (4-hydroxy-3-methoxy benzylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O_3$, yield (89%), red. M.P.–160°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3443.78 (N-H elongation), 3287.31 (Ar. O-H elongation), 3046.79 (Ar. C-H elongation), 2834.79 (C-O-CH_3 elongation), 1661.36 (C=O elongation), 1630.92 (Ar.C=C elongation), 1524.23 (C=N elongation), 1351.61 (C-N elongation), 1023.59 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.27 (1H, s) 9.93 (1H, s), 8.97 (1H, d) 8.56 (1H, s), 8.39 (1H,

s), 8.22 (1H, s), 7.60 (2H, m), 7.52 (2H, m), 7.36 (2H, m), 6.89 (2H, m), 6.79 (2H, m), 6.50 (1H, s), 4.66 (1H, s), 4.60 (3H, S). ESI MS of $C_{21}H_{19}N_3O_3$ found is 361.39.

Compound IV g

2-phenylaminobenzoic acid (2-bromo-3-hydroxy-4-methoxybenzylidene) hydrazide: Molecular formula: $C_{21}H_{18}BrBRN_3O_3$ Yield (86%), light yellow. M.P.–235°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3421.61 (N-H elongation), 3283.28 (Ar. O-H elongation), 3041.36 (Ar. C-H elongation), 2830.38 (C-O-CH_3 elongation), 1659.42 (C=O elongation), 1629.59 (Ar.C=C elongation), 1528.38 (C=N elongation), 1362.44 (C-N elongation), 1020.42 (Ar. C-C elongation), 648.98 (Ar. C-Br elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.368 (s, 1H, NH-N), 9.864 (s, 1H, Ar. OH), 8.989 (s, 1H, N=CH), 8.989 (s, 1H, N=CH), 8.021–8.366 (d, 2H, Ar. H), 7.428–7.862 (m, 4H, Ar. H), 6.261–6.787 (m, 5H, Ar. H), 4.686 (s, 1H, NH), δ 3.648 (s, 3H, OCH_3), ESI MS of $C_{21}H_{18}BrN_3O_3$ found is 440.29.

Compound IV h

2-phenylaminobenzoic acid (4-dimethylamino benzylidene) hydrazide: Molecular formula: $C_{22}H_{22}N_4O$, Yield (92%), yellow, M.P.–242°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3438.51 (N-H elongation), 3046.62 (Ar. C-H elongation), 1661.12 (C=O elongation), 1639.24 (Ar.C=C elongation), 1523.94 (C=N elongation), 1317.92 (C-N elongation), 1024.44 (Ar. C-C elongation), 739.29 (C-N elongation 2^0 amine). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.369 (s, 1H, NH-N). 8.913 (s, 1H, N=CH), 8.101–8.468 (dd, J=9.15, 4H, Ar. H), 7.379–7.746 (m, 4H, Ar. H), 6.366–6.848 (m, 5H, Ar. H), 4.789 (s, 1H, NH), 3.016 (ds, 6H, N-$(CH_3)_2$), ESI MS of $C_{22}H_{22}N_4O$ found is 358.18.

Compound IV i

2-Phenylaminobenzoic acid (3hydroxy-4-methoxy benzylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O_3$, yield (88%), brown. M.P.–206°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3441.87 (N-H elongation), 3281.39 (Ar. O-H elongation), 3040.97 (Ar. C-H elongation), 2828.32 (C-O-CH_3 elongation), 1660.92 (C=O elongation), 1628.29 (Ar.C=C elongation), 1526.46 (C=N elongation), 1363.65 (C-N elongation), 1020.66 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.348 (s, 1H, NH-N), 9.896 (s, 1H, Ar. OH), 8.918 (s, 1H, N=CH), 8.696 (s, 1H, Ar. H), 8.131-8.261 (d, 2H, Ar. H), 7.481-7.868 (m, 4H, Ar. H) 6.332-6.816 (m, 5H, Ar. H), 4.743 (s, 1H, NH), 3.611 (s, 3H, OCH_3). ESI MS of $C_{21}H_{19}N_3O_3$ found is 361.26.

Compound IV j

2-phenylaminobenzoic acid (3,5di-tertbutyl-2-hydroxy benzylidene)hydrazide: Molecular formula: $C_{28}H_{33}N_3O_2$ Yield (75%), light yellow, M.P.–196°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3423.82 (N-H elongation), 3280.89 (Ar. O-H elongation), 3039.49 (Ar. C-H elongation), 2876.49 (Aliphatic C-H elongation), 1657.17 (C=O elongation), 1627.95 (Ar.C=C elongation), 1525.52 (C=N elongation), 1368.21 (C-N elongation), 1019.29 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.352 (s, 1H, NH-N), 9.818 (s, 1H, Ar. OH), 8.996 (s, 1H, N=CH), 8.789 (s, 1H, Ar. H), 8.585 (s, 1H, Ar. H), 7.332–7.722 (m, 4H, Ar. H), 6.341–6.818 (m, 5H, Ar. H), 4.717 (s, 1H, NH), 2.348 (ts, 9H, C-$(CH_3)_3$), 2.089 (ts, 9H, C-$(CH_3)_3$). ESI MS of $C_{28}H_{33}N_3O_2$ found is 329.15.

Compound IV k

2-phenylaminobenzoic acid (1-phenylethylidene) hydrazide: Molecular formula: $C_{21}H_{19}N_3O$ yield (75%), light yellow, M.P.–196°C. FTIR (KBr) $\nu_{max}cm^{-1}$: 3420.87 (N-H elongation), 3038.46 (Ar. C-H elongation), 2875.65 (Aliphatic C-H elongation), 1656.56 (C=O elongation), 1619.59 (Ar.C=C elongation), 1528.18 (C=N elongation), 1371.78 (C-N elongation), 1017.96 (Ar. C-C elongation). ^{1}H-NMR (500 MHz, Chloroform-*d*): δ(in ppm): 11.391 (s, 1H, NH-N), 8.768 (s, 1H, N=CH), 8.022-8.551 (m, 5H, Ar. H), 7.369–7.791 (m, 4H, Ar. H), 6.331–6.818 (m, 5H, Ar. H), 4.614 (s, 1H, NH), 2.161 (s, 3H, C-CH_3). ESI MS of $C_{20}H_{16}ClN_3O$ found is 443.26.

Experimental

Pharmacological Activity

Antibacterial activity

All the newly synthesized derivatives of arylidene hydrazide 4(a-k) were assayed for *in-vitro* antibacterial activity by cup borer diffusion method.[22-24] Gatifloxacin was used as a standard drug to check antibacterial property against pathogenic representative Gram-positive *Bacillus subtilis* (MTCC441), *Bacillus cereus* (MTCC-7190) and Gram-negative *Escherichia coli* (MTCC-40). These bacterial strains selected to check antibacterial property of synthesized compounds are most common and easily available and were grown individually in Luria Broth medium and the cell separation was spread over the surface of Mueller-Hilton agar plates using sterile spreaders. The plates were then allowed to dry and a sterile well borer of 6 mm diameter was used to cut uniform wells in the agar. After incubation at 37°C for 24 h, the plates were observed for the development of a zone of inhibition (ZOI) in diameter around the well. The antibacterial activity was then evaluated by measuring the diameter of observed clear ZOI. The inhibition zones of the test compounds were measured and compared with standard.

Antifungal activity

Antifungal activity was screened against two pathogenic fungi infecting plant, namely, *Candida albicans* and *Aspergillus fumigates* (ATTC 9197).[25] Amphotericin-B was used as a standard antifungal agent. For fungal stains, *C. albicans* and *A. fumigates*, potato dextrose agar (PDA) was used as a medium to grow selected fungi. Glass Petri dishes were sterilized in an oven and melted PDA medium was poured into each Petri dish in an aseptic condition. After solidification of the medium, small portions of mycelium of each fungus were spread carefully over the center of each PDA plate with the help of a sterilized needle. Then, each fungus was transferred to a series of PDA plates. The PDA plates were then incubated at 25 ± 2°C and after 5 days of incubation fungal growth were observed. Plates were inverted and incubated at 37°C for 72 h. The ZOI was measured to assess for pathogenicity of tested compounds. The experiment was repeated thrice to avoid the error confirming the validation. After the incubation period, the diameter of inhibition zone was measured and documented as an indicator for the activity of the compounds (4a-k).

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity was determined by observing the bleaching of the purple colored methanol solution of DPPH using radical scavenging activity.[26,27] The compounds which have

hydrogen atom or electron donation ability only react with free radicals produced by the DPPH. A 1 mL each compound with four concentrations was prepared (25, 50, 75, and 100 µg/mL) in methanol and added to 4 mL of 0.004% w/v methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used as the standard. The percentage of inhibition of free radical production from DPPH was calculated by the following equation. Tests were carried out in triplicate. The % scavenging of DPPH radical was calculated by the following equation.

$$\text{DPPH Scavanging effect}(\%) = [\frac{Ac - As}{Ac}] \times 100$$

$$\text{IC50 in } \mu g/mL = \frac{50 \times 100}{\%Inhibition}$$

$$\text{IC50 in } \mu mol/mL = \frac{\%\text{ of IC50}}{MW\ of\ the\ compound}$$

Where, A_c is the absorbance of control (DPPH solution without the test compound) and A_s is the absorbance of control (DPPH solution with the test compound solution).

Nitric oxide (NO) scavenging assay

Sodium nitroprusside[28,29] (5 µM) in phosphate buffer pH 7.4 was incubated with different concentrations (25, 50, 75, and 100 µg/mL) of test compounds dissolved in methanol and tubes were incubated at 25°C for 2 h. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 mL of incubation solution was taken and diluted with 0.5 mL of Griess reagent (1% sulfanilamide, 0.1% N naphthylethylenediamine dihydrochloride, and 2% phosphoric acid dissolved in distilled water). The chromophore absorption was read at 546 nm which was after diazotization of nitrite with sulfanilamide and chlorides. The chromophore formed after diazotization of nitrite with sulfanilamide and subsequent N naphthylethylenediamine dihydrochloride was read at 546 nm. The experiment was repeated in triplicate. NO scavenging activity was calculated by the following equation is evaluated below.

$$\%\text{ of scavanging} = [\frac{Ac - As}{Ac}] \times 100$$

Where, A_c is the absorbance of the standard and A_s is the absorbance in the presence of the sample and standard.

Hydrogen peroxide (H_2O_2) scavenging assay

The H_2O_2[30] scavenging ability of the title compounds was determined according to the method of solution of H_2O_2 (40 mm) was prepared in phosphate buffer (pH 7.4). A 25, 50, 75, and 100 µg/mL concentrations of the test compounds in 3.4 mL of phosphate buffer were added to H_2O_2 solution of 0.6 mL, 40 mm. The absorbance value of the reaction mixture was recorded at 230 nm. Ascorbic acid was used as a standard. The percentage of scavenging of H_2O_2 was calculated using the following equation.

$$\%\text{ of scavanging} = [\frac{Ac - As}{Ac}] \times 100$$

Where, A_c is the absorbance of the standard and A_s is the absorbance in the presence of the sample and standard.

Table 1: Inhibition zone (diameter) in mm of synthesized compounds (4a-k) tested bacterial strains by agar well diffusion method

Name of the compounds	*Escherichia coli* (µg/mL)			*Bacillus subtilis* (µg/mL)			*Bacillus cereus* (µg/mL)		
	50	*100*	*150*	*200*	*50*	*100*	*150*	*200*	*50*	*100*	*150*	*200*
4a	6.71±0.12	9.21±0.14	16.34±0.11	18.54±0.19	7.21±0.32	13.21±0.5	24.37±0.05	25.71±0.12	7.71±0.12	17.16±0.21	22.54±0.19	23.18±0.13
4b	06±0.4	9.91±0.10	23.05±0.28	25.43±0.33	07±0.27	11.57±0.758	28.30±0.03	26±0.4	5±0.4	19.1±0.32	25.43±0.33	29.33±0.11
4c	7±0.19	10.22±0.34	17.24±0.21	28.29±0.39	7.5±0.278	13.22±0.756	25.55±0.23	425±0.19	5±0.19	15.61±0.11	27.29±0.39	27.03±0.14
4d	7.6±0.11	11.93±0.41	20.26±0.41	29.42±0.11	7.12±0.89	15.±0.41	19.56±0.06	21±0.11	9±0.11	13.29±0.14	27.42±0.11	29.83±0.19
4e	6.7±0.2	9.1±1.02	33.5±0.31	35.9±0.23	6.37±0.47	14.1±1.542	20.5±0.19	26±0.2	9.5±0.2	12.33±0.33	15.2±0.23	16.6±0.22
4f	4.9±0.3	11.2±0.03	14.0±0.31	18.3±0.12	4.37±0.94	15.2±0.53	25.5±0.14	24.1±0.3	8.1±0.3	17.39±0.12	24.3±0.12	29.1±0.20
4g	7.8±0.4	9.12±0.15	17.53±0.65	27.8±0.16	7.368±1.5	11.12±0.87	21.8±0.03	24.8±0.4	9.8±0.4	24.09±0.22	36.8±0.16	36.5±0.19
4h	9.1±0.15	13.91±0.24	27.20±0.20	29.07±015	10.587±0.665	10.91±0.35	28.12±0.14	39±0.15	12±0.15	18.03±0.21	24.01±0.15	29.33±0.12
4i	7.7±0.32	14.22±0.25	20.16±0.08	25.82±0.16	7.573±0.736	11.75±0.27	21.82±0.12	27±0.32	11±0.32	25.82±0.20	35.82±0.16	34.61±0.13
4j	8.76±0.20	11.12±0.17	18.95±0.15	21.32±0.21	9.56±0.7	21.14±0.42	24.26±0.19	32±0.20	12±0.20	15.94±0.19	21.32±0.21	14.69±0.13
Std	7.25±015	10.18±0.17	26.29±0.15	28.14±0.10	6.786±8.6	12.18±0.532	23.33±0.12	25±0.15	22±0.15	25.17±0.22	30.14±0.10	38.29±0.15

Std: Gatifloxacin, *antibacterial activity was carried out at 50, 100, 150, and 200 µg/mL

Table 2: *In vitro* evaluation of antifungal activity of the synthesized compounds 4(a-k)

Name of the compounds	*Candida albicans* (μg/mL)				*Aspergillus fumigates* (μg/mL)			
	50	100	150	200	50	100	150	200
4a	12.126±0.5	22.145±2.0	25.58±3.33	26.458±8.0	11.35±0.5	21.5458±1.5	26.156±2.9	29.145±6.4
4b	-	23.45±0.36	26.58±0.8	28.12±1.0	11.56±0.2	22.528±1.1	28.15±1.3	30.145±1.8
4c	12.652±1.0	25.56±3.43	27.6±6.25	29.15±0.1	10.26±1.0	23.05±2.2	23.15±4.1	24.59±7.5
4d	13.54±0.1	21.56±1.93	26.58±1.8	27.15±3.0	16.15±0.2	24.58±1.1	29.456±2.3	30.12±4.0
4e	-	-	24.55±0.1	26.4580.3	-	23.52±0.1	27.26±0.3	31.05±0.8
4f	-	21.96±0.28	24.25±0.3	28.156±0.5	-	8.15±0.1	16.15±0.3	16.158±0.6
4g	-	-	29.27±0.1	34.51±0.2	-	-	10±2	13±4
4h	-	26.54±0.36	27.55±1.1	30.11±2.0	12.2±0.1	23.14±0.5	33.15±0.8	39.15±1.4
4i	-	23.5±0.51	27.15±1.3	30.125±2.2	16.15±0.1	21.59±0.8	26.145±1.3	27.458±3.5
4j	11.59±0.24	23.26±1.07	24.9±1.28	31.15±3.3	16.45±1.0	20.15±1.3	22.54±3.2	27.6912±4.3
Std	12.64±2.59	12.556±3.82	20.58±6.06	27.15±12.13	13.77±1.06	16.05±2.85	19.14±5.60	21.89±11.87

Std: Amphotericin-B, *antifungal activity was carried out at concentrations 50, 100, 150, and 200 μg/Ml

Table 3: The *in vitro* antioxidant activity of title compounds 7(a-j) by DPPH method

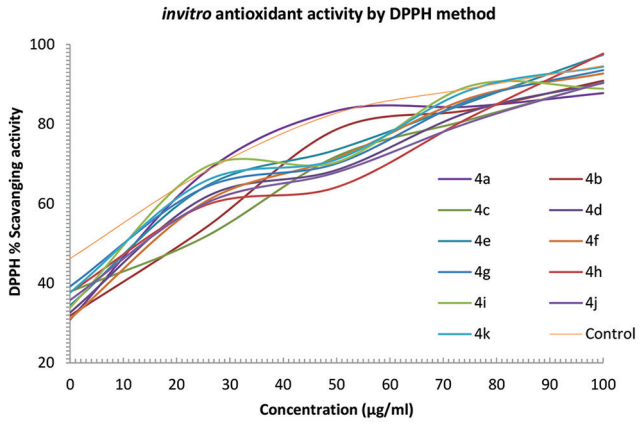
Sample code	Concentration (μg/ml)					IC50 (μg/mL)
	0	25	50	75	100	
4a	30.89	67.56	83.34	84.4	87.78	10
4b	31.88	53.56	78.67	83.55	90.89	22.8
4c	37.9	51.45	71.89	81.12	90.4	24.6
4d	32.65	61.343	68.45	83.09	90.34	12.05
4e	34.43	64	73.56	85.56	97.43	23.54
4f	31.09	60.21	71.4	86.54	92.71	15.67
4g	39.21	63.89	70.09	86.02	93.56	16.39
4h	37.77	59.42	64.14	81.67	97.7	9
4i	33.9	68.88	70.45	89.57	88.89	12.6
4j	35.78	59.87	67.89	80.47	90.5	14
4k	37.67	65.45	71.12	88.56	94.43	15
Control	46.21	67.8	82.78	88.98	94.67	4.8

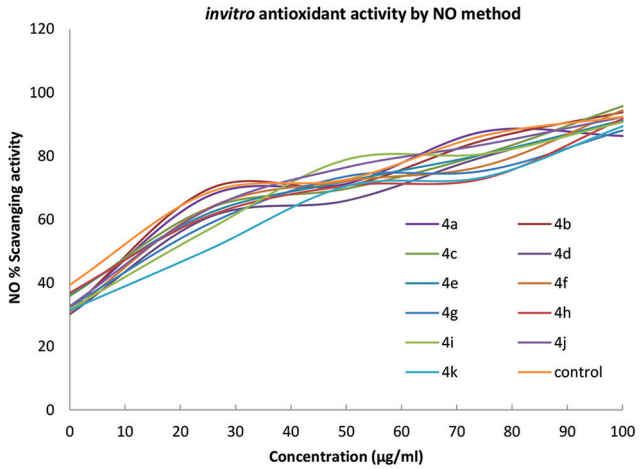
Values were the means of three replicates±SD

Table 4: The *in vitro* antioxidant activity of title compounds (4a-k) by NO method

Sample code	Concentration (μg/ml)					IC50 (μg/mL)
	0	25	50	75	100	
4a	35.32	63.34	72.3	80.45	89.54	13.5
4b	32.09	60.35	70.45	76.21	91.23	15.49
4c	31	65.45	68.45	74.9	93.4	13.74
4d	29.88	62.37	69.6	75.23	92.5	15.64
4e	28.74	58.67	71.13	75.03	92.31	16.8
4f	31.78	61.11	76.4	78.44	90.34	14.64
4g	34.98	60.56	73.89	71.09	91.14	13.54
4h	36.01	62.46	74.98	78.56	87.44	12.64
4i	31.19	64.56	70.65	73.33	91.43	15.57
4j	34.9	62.36	71.14	80.44	90.33	15.64
4k	31.14	60.35	75.9	81.24	89.94	14.91
Control	40.21	68.56	75.9	86.5	91.73	8.6

Values were the means of three replicates±SD





Pharmacological Activity

Antibacterial activity

The newly synthesized arylidene hydrazones 4(a-k) were screened against two Gram-positive bacteria such as *B. subtilis* MTCC 441 and *B. cereus* MTCC 7190 one Gram-negative bacteria such as *E. coli*, MTCC 40 by the agar well diffusion method. The ZOI of the tested compounds was compared with standard. The bio-screening data revealed that 4b, 4f, 4g, 4h, and 4j moiety exhibited good antibacterial activity against *B. subtilis, B. cereus,* and also *E. coli*. The bacterial screening was performed in triplicate and their mean values were taken for antibacterial analysis which is depicted in Table 1. The title compounds showed their potential to serve as a good platform for further investigation to discover new derivatives having an improved overall biological profile.

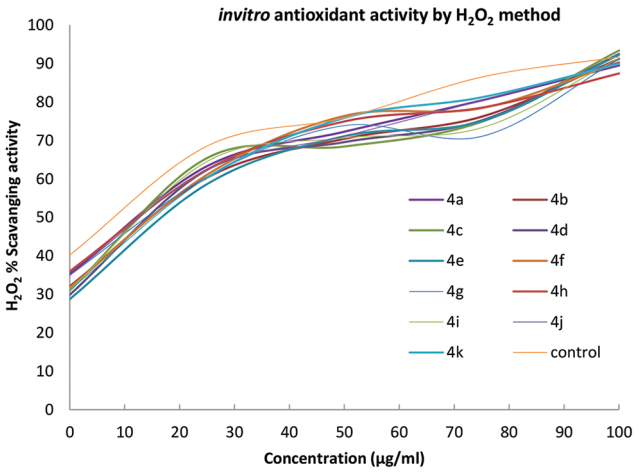
Antifungal activity

The antifungal activity of the newly synthesized title compounds 4(a-k) was screened against two pathogenic mold fungi, namely, *C. albicans* and *A. fumigates* (ATTC 9197)[4] and amphotericin-B was used as the standard. Afterward, the plates were incubated at 37°C for 72 h. Compounds 4a and 4c moiety exhibited good antifungal activity, as shown in Table 2.

Table 5: The *in vitro* antioxidant activity of 4(a-k) compounds by H_2O_2 method

Sample code	*Concentration (µg/ml)*					*IC50 (µg/mL)*
	0	*25*	*50*	*75*	*100*	
4a	31.69	67.3	71.2	87.7	86.3	13.24
4b	31.34	69.45	70.56	84.98	93.76	11.24
4c	35.87	63.45	69.67	80.66	95.67	14.982
4d	30.12	60.58	65.89	79.78	90.8	15.42
4e	36.45	61.83	71.99	80.54	91.24	13.76
4f	30.72	63.49	72.09	76.89	94.36	14.83
4g	32.51	58.43	73.56	75.34	88.04	16.6
4h	36.92	60.99	70.6	72.9	91.67	15.4
4i	31.77	56.7	78.83	80.67	90.67	18.31
4j	32.67	63.2	76.4	83.6	92.26	15.13
4k	31.44	50.4	70.46	73.34	89.32	25
Control	39.42	68.4	72.56	86.45	92.33	9

Values were the means of three replicates±SD



Antioxidant activity

The free radical scavenging activity by this method was determined by observing the bleaching of the purple-colored methanol solution of DPPH. The newly synthesized arylidene hydrazides (4a-k) derivatives were tested at different concentrations (25, 50, 75, and 100 µg/mL) and showed potential to moderate activity. Among the compounds 4a and 4c exhibited good antioxidant activity, the reason might be the presence of electron-withdrawing groups (fluoro and bromo groups) on phenyl ring of sulfonyl derivatives, as shown in Table 3. In NO method,[31] compounds 4a and 4c exhibited good activity, as shown in Table 4. In H_2O_2 method, 4b, 4e, 4g, 4h, and 4j having good activity because the presence of electron-withdrawing groups (chloro and nitro groups) on phenyl ring of sulfonyl and carbamate derivatives, as shown in Table 5. The wide variations in free radical scavenging activities may be attributed to the various substituents on the phenyl ring.

Results and Discussion

In this work, ortho chloro benzoic acid on reaction with aniline through Ullmann Reaction gave N-phenyl anthranilic acid (1). The esterification product of N-phenyl anthranilic acid (2) followed by reaction with hydrazine hydrate yielded 2-phenyl amino benzoic acid hydrazide (3). These hydrazides were give arylidine hydrazide as title compounds with different aldehydes. The presence of 11.391 for singlet NH-N (IVk) showed that the azomethine ring, whereas all the compounds showed approx. this rang for ^{1}H-NMR.

All newly synthesized derivatives were tested for antimicrobial activity as well as antioxidant activity. The synthesized compounds showed mild-to-moderate antibacterial activity against Gram-positive *B. subtilis* (MTCC441), *B. cereus* (MTCC-7190) and Gram-negative *E. coli* and antifungal activity against *C. albicans* and *A. fumigates* (ATTC 9197). The bio-screening data revealed that 4b, 4f, 4g, 4h, and 4j moiety exhibited good antibacterial activity against all because of its electronegativity. The compounds 4a and 4c were showed good antifungal activity because they are having electro-donating group. Among the compounds, 4a and 4c exhibited good antioxidant activity.

Conclusion

We herein describe the multicomponent, synthesis of some novel Arylidene hydrazides derivatives in short reaction times and from easily affordable starting material and their antibacterial activity and antifungal activity. All the synthesised compounds are novel structure. The result of antimicrobial study indicated that the presence of electronegative, halogen moiety in aromatic ring improved antibacterial activity, whereas the presence of nitro group improved antifungal activity of substituted hydrazides.

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