



Synthesis of Some Hippuric Acid Substrate Linked Novel Pyrazoles as Antimicrobial Agents

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Escalating resistance of microorganisms to the currently accessible antimicrobial drugs has forced to synthesize some novel biologically active compounds as efficient alternates *via* economical substrates. Hence, hippuric acid was used as one of the starting materials to synthesize pyrazole derivatives. All the synthesized compounds were characterized by IR, NMR (^1H & ^{13}C) and mass spectral data. The antimicrobial potential of synthesized compounds has been explored against four bacterial and two fungal strains. Among the 12 compounds, 3 compounds **8j**, **8k** and **8l** were found to exhibit prominent antimicrobial potential as compared with the standards ciprofloxacin and amphotericin-B.

Keywords: Hippuric acid, Pyrazoles, Antimicrobial agent, Ciprofloxacin, Amphotericin-B.

INTRODUCTION

“Pyrazole” the term coined by German Chemist Ludwig Knorr, is a heterocyclic compound containing two nitrogen atoms at adjacent positions and two endocyclic double bonds. Pyrazole derivatives have shown great potential in agrochemicals [1-5] and pharmaceutical industry [6-8] as herbicides and active pharmaceuticals. German Chemist Hans von Pechmann was the first to synthesize pyrazole from acetylene and diazomethane and in year 1959 the first natural pyrazole, 1-pyrazolyl-alanine was isolated from seeds of watermelons. Since the mid of 19th century, pyrazoles have attracted considerable attention in different fields of science. Investigations of its physical, chemical, and biological properties yielded promising informations [9-16]. Number of substituted pyrazole derivatives were acknowledged to possess a wide range of biological activities *e.g.*, antibacterial [1], antitumor [6], antitubercular [7], anti-inflammatory [9], antifungal, antiviral, antiparasitic, insecticidal and antioxidant [11-16], *etc.*

Mahapatra *et al.* [17] synthesized a potent anti-inflammatory pyrazole derivative using hippuric acid as starting material. Further Afonso *et al.* [18] reported an oxazolone (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one again using hippuric acid as a substrate with aldehyde in presence of sodium acetate and acetic anhydride. Nagamani *et al.* [19] synthesized some

new 4-arylbenzylidene-2-phenyl-5-oxazolones by the condensation of hippuric acid and aromatic aldehydes. The synthesized compounds exhibited an excellent level of activity against *E. coli*. Ahmed [20] synthesized different oxazol-5(4*H*)-ones by the condensation of *o*-hydroxy hippuric acid with aromatic aldehydes in presence of catalytic amount of acetic anhydride and sodium acetate which exhibited excellent antimicrobial activity.

In view of the above, lead was taken for the preparation of some novel pyrazole derivatives containing hippuric acid moiety as potent antimicrobial agents.

EXPERIMENTAL

The chemicals were purchased from commercial suppliers and used without further purification. Melting points were determined in open glass capillaries in melting point apparatus and uncorrected. IR spectra were recorded on Perkin-Elmer 157 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 400 MHz spectrophotometer using DMSO- d_6 as a solvent. Mass spectra were recorded on UPLC-MS.

Synthesis of *N*-(2-hydrazinyl-2-oxoethyl)benzamide: Ethyl-2-benzamidoacetate (**2**) (0.01 mol) was dissolved in absolute ethanol (40 mL) and hydrazine hydrate (0.02 mol) was added dropwise to the reaction mixture with constant stirring.

The resulting reaction mixture was refluxed for 6 h. Excess solvent was distilled off and the contents were cooled to room temperature. The solid product thus obtained was filtered, washed with water and dried. Further, it was recrystallized from ethanol (**Scheme-I**).

Synthesis of 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehydes (7a-f): The solution of substituted acetophenone (0.042 mol) in ethanol (10 mL) was added to an ethanolic solution of phenylhydrazine (0.050 mol in 10 mL ethanol) at room temperature. One drop of conc. H₂SO₄ was added and the mixture was stirred, refluxed for 55 min. The excess solvent was distilled off and the reaction mixture was cooled to 20-25 °C. The crude solid thus obtained was filtered, dried and recrystallized from ethanol.

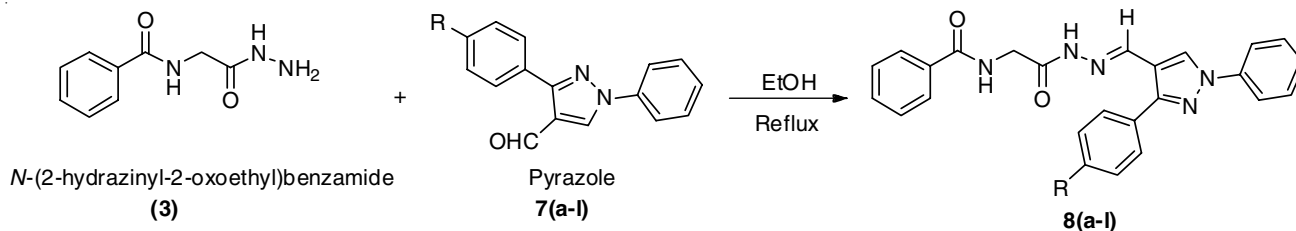
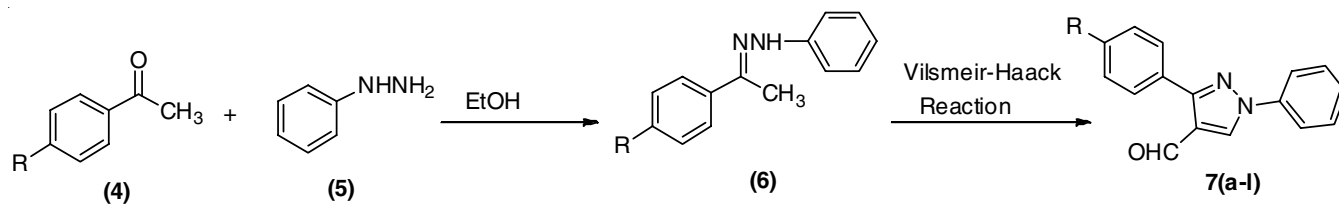
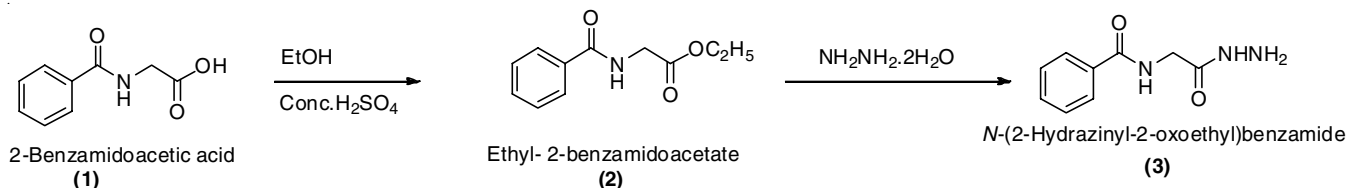
To the cold solution of DMF (15 mL) and acetophenone phenylhydrazone (0.024 mol), Vilsmeier-Haack reagent prepared from dimethylformamide (0.071 mol) and POCl₃ (0.071 mol) was added in small lots within 30 min at 0-5 °C. The reaction mixture was stirred at 60-65 °C for 5 h and finally poured into ice-cold water. The precipitates obtained on neutralization with

sodium bicarbonate were filtered, washed with water and recrystallized from ethanol (**Scheme-II**).

Further, the synthesized derivatives **3** and **7(a-f)** were refluxed in 25 mL ethanol for 3 h. The reaction progress was monitored by TLC using 20 % methanol-chloroform (1:4) as a solvent system. The white solid thus obtained was filtered, washed and recrystallized from ethanol (**Scheme-III**).

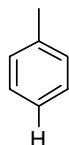
Spectral data

Compound 8a: Colour: Brownish white, m.p.: 144 °C. Elemental analysis calcd. (found) %: C 77.41 (72.29); H 4.83 (3.69); N 11.29 (8.24); O 6.45 (3.40). IR (KBr, ν_{\max} , cm⁻¹): 3354 (N-H *str.*), 1668 (CO *str.*), 1596, 1483 (C=C *str.*). ¹H NMR (400 MHz: DMSO-*d*₆, δ_{H} , ppm): 8.66 (t, 1H, NH), 8.35 (t, 1H, NH), 9.03 (s, 1H, Pyrazole-H), 8.35 (s, 1H, -CH), 4.38 (s, 2H, -CH₂), 8.03 (m, 2H, 1,5), 7.63 (m, 2H, 2,4), 7.70 (m, 1H, 3), 7.62 (m, 2H, 1',5'), 7.58 (m, 2H, 2',4'), 7.45 (m, 1H, 3'), 7.79 (m, 2H, 1'',5''), 7.51 (m, 2H, 2'',4''), 7.41 (m, 1H, 3''). ¹³C NMR (400 MHz: DMSO-*d*₆, δ_{C} , ppm): 39.68, 39.89, 116.89,

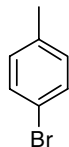


where R =

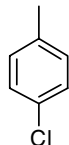
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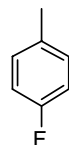
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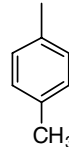
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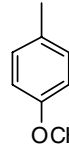
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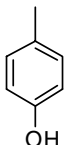
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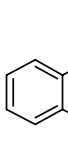
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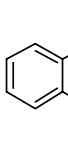
8g =



8h =



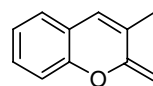
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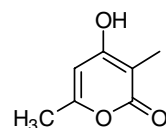
8j =



8k =



8l =



Scheme-III

118.64, 127.22, 127.33, 127.96, 128.30, 128.42, 128.55, 128.73, 129.56, 131.29, 131.38, 131.97, 132.18, 133.82, 134.11, 136.34, 139.01, 139.84, 151.16, 151.78, 165.17, 166.51, 169.84. Mass (m/z): Observed [M]⁺: 423.

Compound 8b: Colour: White, m.p.: 173 °C. Elemental analysis calcd. (found) %: C 58.71 (52.68); H 3.66 (2.62); N 8.56 (4.52); O 4.89 (4.84); Br 24.46 (20.42). IR (KBr, ν_{\max} , cm^{-1}): 3357 (N-H *str.*), 1669 (CO *str.*), 1594, 1485 (C=C *str.*), 654 (C-Br *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.63(t, 1H, NH), 8.37(t, 1H, NH), 9.07(s, 1H, Pyrazole-H), 8.38(s, 1H, -CH), 4.36(s, 2H, -CH₂), 8.01 (m, 2H, 1,5), 7.66 (m, 2H, 2,4), 7.73 (m, 1H, 3), 7.65 (m, 2H, 1',5'), 7.57 (m, 2H, 2',4'), 7.47 (m, 1H, 3'), 7.78 (m, 2H, 1'',5''), 7.66(m, 2H, 2'',4''). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 41.34, 42.39, 117.35, 117.43, 119.18, 119.31, 122.42, 127.53, 127.83, 128.81, 129.05, 130.93, 131.81, 131.98, 132.11, 134.59, 136.63, 139.40, 140.03, 150.34, 150.90, 165.73, 167.05, 170.46. Mass (m/z): Observed [M]⁺: 502.

Compound 8c: Colour: White, m.p.: 163 °C. Elemental analysis calcd. (found) %: C 68.08 (63.02); H 4.25 (2.21); N 9.92 (5.84); O 5.67 (3.62); Cl 12.58 (10.13). IR (KBr, ν_{\max} , cm^{-1}): 3353 (N-H *str.*), 1665 (CO *str.*), 1593,1487 (C=C *str.*) 710 (C-Cl *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.69 (t, 1H, NH), 8.33 (t, 1H, NH), 9.07 (s, 1H, Pyrazole-H), 8.33 (s, 1H, -CH), 4.38 (s, 2H, -CH₂), 8.02 (m, 2H, 1,5), 7.67 (m, 2H, 2,4), 7.73 (m, 1H, 3), 7.65 (m, 2H, 1',5'), 7.53 (m, 2H, 2',4'), 7.47 (m, 1H, 3'), 7.98(m, 2H, 1'',5''), 7.55(m, 2H, 2'', 4''). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 41.45, 117.36, 117.43, 119.17, 119.31, 127.72, 127.83, 128.82, 129.06, 129.19, 130.67, 131.59, 139.40, 150.28, 150.78, 150.85, 165.70, 167.02, 170.45. Mass (m/z): Observed [M]⁺: 457

Compound 8d: Colour: White, m.p.: 160 °C. Elemental analysis calcd. (found) %: C 72.18 (68.09), H 4.51 (2.46), N 10.52 (6.46), O 6.01 (3.01), F 7.14 (6.08). IR (KBr, ν_{\max} , cm^{-1}): 3359 (N-H *str.*), 1663 (CO *str.*), 1595,1486 (C=C *str.*)1250 (C-F *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.65 (t, 1H, NH), 8.39 (t, 1H, NH), 9.08 (s, 1H, Pyrazole-H), 8.37 (s, 1H, -CH), 4.33 (s, 2H, -CH₂), 8.05 (m, 2H, 1, 5), 7.67 (m, 2H, 2,4), 7.78 (m, 1H, 3), 7.65 (m, 2H, 1',5'), 7.56 (m, 2H, 2',4'), 7.46 (m, 1H, 3'), 8.15 (m, 2H, 1'',5''), 7.30 (m, 2H, 2'',4''). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 41.35, 42.41, 115.81, 115.98, 116.02, 116.20, 117.22, 117.31, 119.12, 119.27, 127.42, 127.72, 127.83, 128.79, 128.87, 130.06, 131.05, 131.14, 136.74, 139.45, 150.60, 151.19, 161.54, 165.69, 167.01, 170.43 Mass (m/z): : Observed [M]⁺: 441

Compound 8e: Colour: Off white, m.p.: 62 °C. Elemental analysis calcd. (found) %: C 77.86 (73.39); H 5.34 (2.79); N 10.68 (6.24); O 6.10 (3.40). IR (KBr, ν_{\max} , cm^{-1}): 3353 (N-H *str.*), 1663 (CO *str.*), 1596,1487 (C=C *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.62 (t, 1H, NH), 8.34 (t, 1H, NH), 9.07 (s, 1H, Pyrazole-H), 8.37 (s, 1H, -CH), 4.33 (s, 2H, -CH₂), 8.05 (m, 2H, 1,5), 7.66 (m, 2H, 2,4), 7.75 (m, 1H, 3), 7.68 (m, 2H, 1',5'), 7.55 (m, 2H, 2',4'), 7.47 (m, 1H, 3'), 7.67 (m, 2H, 1'',5''), 7.29 (m, 2H, 2'',4''), 2.34 (m, 3H, -CH₃). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 39.74, 39.95, 40.16, 40.37, 40.58, 41.39, 42.40, 117.15, 117.30, 119.11, 119.24, 127.72, 127.84, 128.76, 128.81, 129.66, 129.79, 130.05, 138.48, 139.53, 151.73, 152.33, 165.68, 167.04, 170.44. Mass (m/z): Observed [M]⁺: 437

Compound 8f: Colour: Whitish brown, m.p.: 136 °C. Elemental analysis calcd. (found) %: 73.38 (68.38), H 5.03 (2.99), N 10.07 (8.03), O 11.50 (7.45). IR (KBr, ν_{\max} , cm^{-1}): 3353 (N-H *str.*),1668 (CO *str.*), 1592,1483 (C=C *str.*) 1240 (C-O *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.68 (t, 1H, NH), 8.39 (t, 1H, NH), 9.02 (s, 1H, Pyrazole-H), 8.35 (s, 1H, -CH), 4.37 (s, 2H, -CH₂), 8.07 (m, 2H, 1,5), 7.67 (m, 2H, 2,4), 7.70 (m, 1H, 3), 7.66 (m, 2H, 1',5'), 7.56(m, 2H, 2',4'), 7.45(m, 1H, 3'), 7.54 (m, 2H, 1'',5''), 7.05 (m, 2H, 2'', 4''), 3.83 (m, 3H, -OCH₃). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 40.57, 41.40, 55.69, 114.47, 116.98, 117.11, 119.04, 125.04, 127.71, 128.82, 130.21, 130.25, 134.61, 139.53, 151.52, 152.13, 160.08, 165.66, 170.43. Mass (m/z): Observed [M]⁺: 453.

Compound 8g: Colour: Yellowish brown, m.p.: 88 °C. Elemental analysis calcd. (found) %: C 72.72 (67.72); H 4.54 (3.99); N 10.60 (7.03), O 12.12 (8.45). IR (KBr, ν_{\max} , cm^{-1}): 3500 (O-H *str.*), 3356 (N-H *str.*), 1667 (CO *str.*), 1596,1489 (C=C *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.65 (t, 1H, NH), 8.33 (t, 1H, NH), 9.07(s, 1H, Pyrazole-H), 8.37(s, 1H, -CH), 4.36 (s, 2H, -CH₂), 8.02 (m, 2H, 1,5), 7.65 (m, 2H, 2,4), 7.75 (m, 1H, 3), 7.67 (m, 2H, 1',5'), 7.56 (m, 2H, 2',4'), 7.47 (m, 1H, 3'), 7.49 (m, 2H, 1'', 5''), 6.86 (m, 2H, 2'',4''), 5.35 (m, 1H, -OH). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 41.39, 42.39, 56.52, 115.84, 116.82, 116.94, 119.00, 119.13, 123.20, 123.39, 127.72, 127.83, 128.15, 128.82, 130.24, 131.90, 134.60, 139.56, 140.70, 151.93, 152.55, 158.35, 158.43, 165.65, 167.05, 170.40. Mass (m/z): Observed [M]⁺: 439.

Compound 8h: Colour: Brown, m.p.: 48 °C. Elemental analysis calcd. (found) %: C 80.53 (74.50); H 4.69 (2.63); N 9.39 (6.60); O 5.36 (2.32). IR (KBr, ν_{\max} , cm^{-1}): 3357 (N-H *str.*), 1666 (CO *str.*), 1598,1483 (C=C *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.62 (t, 1H, NH), 8.33 (t, 1H, NH), 9.08 (s, 1H, Pyrazole-H), 8.34 (s, 1H, -CH), 4.39 (s, 2H, -CH₂), 8.05 (m, 2H, 1,5), 7.65 (m, 2H, 2,4), 7.73 (m, 1H, 3), 7.64 (m, 2H, 1',5'), 7.55(m, 2H, 2',4'), 7.44 (m, 1H, 3'), 7.95 (m, 1H, 1''), 7.61 (m, 1H, 2''), 8.04 (m, 1H, 3''), 8.08 (m, 1H, 4''), 7.55 (m, 2H, 5'',6''), 8.55(m, 1H, 7''). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 118.83, 125.40, 125.59, 125.99, 126.28, 126.69, 126.87, 126.951, 127.30, 127.44, 128.26, 128.55, 128.87, 129.11, 129.62, 129.89, 131.24, 131.35, 131.60, 133.33, 133.79, 134.11, 135.69, 139.43, 150.38, 151.37, 165.12, 166.36, 169.86. Mass (m/z): Observed [M]⁺: 473.

Compound 8i: Colour: white, m.p.: 42 °C. Elemental analysis calcd. (found) %: C 80.53 (73.44); H 4.69 (4.69); N 9.39 (9.58); O 5.36 (3.30). IR (KBr, ν_{\max} , cm^{-1}): 3354 (N-H *str.*), 1667 (CO *str.*), 1596,1485 (C=C *str.*). ¹H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.66 (t, 1H, NH), 8.34 (t, 1H, NH), 9.09 (s, 1H, Pyrazole-H), 8.34 (s, 1H, -CH), 4.39 (s, 2H, -CH₂), 8.05(m, 2H, 1,5), 7.67 (m, 2H, 2,4), 7.75 (m, 1H, 3), 7.69(m, 2H, 1',5'), 7.55(m, 2H, 2',4'), 7.46 (m, 1H, 3'), 8.34 (m, 1H, 1''), 8.00 (m, 2H, 2'',5''), 7.59 (m, 2H, 3'',4''), 7.92 (m, 1H, 6''), 7.87 (m, 1H, 7''). ¹³C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 118.75, 125.29, 125.59, 125.99, 126.08, 126.69, 126.87, 126.951, 127.20, 127.44, 128.21, 128.55, 128.87, 129.11, 129.62, 129.89, 131.24, 131.35, 131.60, 133.24, 133.79, 134.11, 135.69, 139.11, 150.38, 151.37, 165.12, 166.47, 169.86. Mass (m/z): Observed [M]⁺: 473.

Compound 8j: Colour: white, m.p.: 65 °C. Elemental analysis calcd. (found) %: C 66.14 (62.12); H 3.93 (3.92); N 11.02 (8.02); O 6.29 (6.23); S 12.59 (8.59). IR (KBr, ν_{\max} , cm^{-1}): 3354 (N-H str.), 1663 (CO str.), 1596, 1488 (C=C str.). ^1H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.63 (t, 1H, NH), 8.39 (t, 1H, NH), 9.05 (s, 1H, Pyrazole-H), 8.38 (s, 1H, -CH), 4.37 (s, 2H, -CH₂), 8.08 (m, 2H, 1,5), 7.69 (m, 2H, 2,4), 7.77 (m, 1H, 3), 7.63 (m, 2H, 1',5'), 7.54 (m, 2H, 2',4'), 7.49 (m, 1H, 3'), 7.56 (m, 1H, 1''), 7.17 (m, 1H, 2''), 7.69 (m, 1H, 3''). ^{13}C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 39.58, 39.79, 39.99, 40.20, 40.41, 40.62, 41.43, 42.40, 116.81, 116.93, 119.13, 127.46, 127.51, 127.73, 127.84, 128.36, 128.80, 130.09, 145.86, 146.13, 165.82, 167.05, 170.44. Mass (m/z): Observed $[\text{M}+1]^+$: 430.

Compound 8k: Colour: Yellowish orange, m.p.: 40 °C. Elemental analysis calcd. (found) %: C 64.86 (60.86); H 4.05 (2.05), N 9.45 (11.02), O 21.62 (21.59). IR (KBr, ν_{\max} , cm^{-1}): 3355 (N-H str.), 1665 (CO str.), 1599, 1483 (C=C str.). ^1H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.67 (t, 1H, NH), 8.33 (t, 1H, NH), 9.09 (s, 1H, Pyrazole-H), 8.33 (s, 1H, -CH), 4.35 (s, 2H, -CH₂), 8.07 (m, 2H, 1,5), 7.67 (m, 2H, 2,4), 7.76 (m, 1H, 3), 7.65 (m, 2H, 1',5'), 7.55 (m, 2H, 2',4'), 7.49 (m, 1H, 3'), 8.05 (m, 1H, 1''), 7.84 (m, 1H, 2''), 7.42 (m, 2H, 3'',5''), 7.65 (m, 1H, 4''). ^{13}C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 40.56, 41.02, 55.37, 116.56, 119.28, 121.52, 125.31, 127.56, 127.65, 127.80, 128.77, 129.38, 130.16, 131.73, 132.83, 134.58, 136.59, 139.37, 143.38, 146.06, 146.79, 153.86, 159.55, 165.62, 166.87, 170.27. Mass (m/z): Observed $[\text{M}]^+$: 491.

Compound 8l: Colour: white, m.p.: 89 °C. Elemental analysis calcd. (found) %: C 72.15 (65.12); H 3.79 (1.79); N 8.86 (4.82); O 15.18 (12.12). IR (KBr, ν_{\max} , cm^{-1}): 3350 (N-H str.), 1670 (CO str.), 1593 (C=C str.). ^1H NMR (400 MHz: DMSO- d_6 , δ_{H} , ppm): 8.68 (t, 1H, NH), 8.38 (t, 1H, NH), 9.01 (s, 1H, Pyrazole-H), 8.35 (s, 1H, -CH), 4.35 (s, 2H, -CH₂), 8.06 (m, 2H, 1,5), 7.66 (m, 2H, 2,4), 7.76 (m, 1H, 3), 7.69 (m, 2H, 1',5'), 7.57 (m, 2H, 2',4'), 7.48 (m, 1H, 3'), 6.77 (m, 1H, 1''), 6.21 (m, 1H, 2''), 3.61 (m, 1H, 3''), 1.82 (m, 3H, -CH₃). ^{13}C NMR (400 MHz: DMSO- d_6 , δ_{C} , ppm): 23.34, 44.62, 45.92, 107.85, 114.23, 119.93, 123.43, 124.92, 126.23, 127.52, 127.69, 128.82, 128.93, 129.24, 129.34, 129.65, 132.13, 134.27, 139.72, 141.34, 143.38, 167.82, 171.43, 189.34, 196.57. Mass (m/z): Observed $[\text{M}]^+$: 471.

Antimicrobial activity: Six microbial strains viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces cerevisiae* were selected on the basis of their clinical importance in human beings. All the microbial cultures were purchased from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. Sub-culturing of bacterial strains was done on nutrient agar, while yeast was carried on malt extract agar plates.

Zone of inhibition: The zone of inhibition of synthesized compounds was measured by the agar well diffusion assay. The inoculum suspensions of the test microorganisms were prepared by using 16 h old cultures adjusted to 10^8 cfu/mL by referring 0.5 McFarland standards. Total 20 mL of agar medium (nutrient agar in case of antibacterial and malt extract agar in case of antifungal) was poured into each petri plate, then plates were swabbed with 100 mL inoculum of the test microorganisms and kept for 15 min for adsorption. Wells were bored into the seeded agar plates using a sterile cork borer of diameter

(8 mm) and were loaded with 100 mL volume of concentration of 4.0 mg/mL of each compound dissolved in dimethylsulfoxide (DMSO). The incubation of all the plates was carried at 37 °C for 24 h. Antimicrobial activity of each compound against the selected organisms was evaluated by measuring the zone of inhibition with zone reader (Hi antibiotic zone scale). Ciprofloxacin and amphotericin-B were used as positive control for bacterial and yeast strains respectively. This procedure was performed in three replicate plates for each organism.

Minimum inhibitory concentration (MIC): MIC is the lowest concentration of an antimicrobial compound that inhibits the visible growth of microorganism after incubation. MIC of the synthesized compounds against bacterial and yeast strains was tested through a modified agar well diffusion method. In this protocol, a two-fold serial dilution of each compound was prepared by first dissolving the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 4-0.0625 mg/mL. A 100 mL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 mL of standardized inoculums (10^8 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC was taken as the lowest concentration of the chemical compounds that inhibit the growth of microbes which was shown by a clear zone of inhibition and recorded for each test organism. Ciprofloxacin and amphotericin-B were used as positive controls, while DMSO was used as a negative control in this investigation.

RESULTS AND DISCUSSION

The synthesis of various pyrazole (*E*)-*N*-(2-(2-((1,4-aryl-phenyl)-1*H*-pyrazol-3-yl)methylene)hydrazinyl)-2-oxoethyl)-benzamide derivatives was achieved in this article. The synthesized compounds were characterized by ^1H NMR, ^{13}C NMR, IR and mass spectral data. The absorption bands for -NH and -C=O stretching vibration appeared in IR spectra of compounds (**8a-l**) at 3454 and 1668 cm^{-1} , respectively. The compounds (**8a-l**) displayed two singlets due to pyrazole 5H and N=CH at δ 9.03 and δ 8.35 ppm, respectively which indicates the formation of hippuric acid moiety linked with pyrazole derivatives. The ^{13}C NMR spectra for compounds **8a-l** displayed signals at δ 151.16, 150.90, 150.85, 151.19, 151.73, 151.52, 151.93, 151.37, 151.37, 146.13, 150.83 and 143.38 ppm, respectively corresponding to pyrazole ring carbon which confirmed the formation of the above mentioned derivatives.

Antimicrobial activity: All the 12 synthesized compounds were tested for their *in vitro* antibacterial and antifungal activity against four bacterial strains and two fungal strains respectively by agar well diffusion method. The results obtained were compared with those of the standard ciprofloxacin and amphotericin-B. The preliminary results were recorded by measuring the inhibition zones (IZ) of bacterial and fungal growth around the wells for each tested compound at 400 $\mu\text{g}/100 \mu\text{L}$. It was observed that most of the compounds were found active against the bacterial as well as fungal strains as mentioned in Table-1.

The results of antibacterial screening revealed that among all the tested compounds, compounds **8j** and **8l** exhibited good inhibitory potential against all the four bacterial and two fungal

TABLE-1
INHIBITION ZONE DIAMETER (mm) OF **8(a-l)** USING AGAR WELL DIFFUSION METHOD at 400 µg/100 µL

Compounds	Gram-negative bacteria		Gram-positive bacteria		Fungi	
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>
	1,3-Diphenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8a)	–	–	–	–	10
3-(4-Bromophenyl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8b)	–	–	–	–	12	13
3-(4-Chlorophenyl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8c)	–	–	–	9	8	9
3-(4-Fluorophenyl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8d)	10	–	10	–	9	8
1-Phenyl-3-(<i>p</i> -tolyl)-1 <i>H</i> -pyrazole-4-carbaldehyde (8e)	–	12	–	–	–	–
3-(4-Methoxyphenyl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8f)	–	10	–	–	–	–
3-(4-Hydroxyphenyl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8g)	–	–	12	10	10	10
3-(Naphthalen-1-yl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8h)	9	9	10	11	9	9
3-(Naphthalen-2-yl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8i)	10	12	10	09	9	9
1-Phenyl-3-(thiophen-2-yl)-1 <i>H</i> -pyrazole-4-carbaldehyde (8j)	12	12	14	14	13	12
3-(4-Hydroxy-6-methyl-2-oxo-2 <i>H</i> -pyran-3-yl)-1 <i>H</i> -pyrazole-4-carbaldehyde (8k)	14	10	9	10	11	11
3-(2-Oxo-2 <i>H</i> -chromen-3-yl)-1-phenyl-1 <i>H</i> -pyrazole-4-carbaldehyde (8l)	15	14	16	14	18	14
Ciprofloxacin	25	26	24	25	–	–
Amphotericin B	–	–	–	–	24	20

–: no activity, *values, including diameter of the well (8 mm), are means of three replicates.

strains. The maximum diameter of the inhibitory zone was 16 mm in bacterial strains and 18 mm in fungal strains given by compound **8l**. The inhibitory potential was 66 % as compared to standard antibacterial ciprofloxacin and 70 % to that of anti-fungal amphotericin-B. Compounds **8h**, **8i** and **8k** showed comparatively lower inhibitory zone diameter as compared to compounds **8j** and **8l**.

Conclusion

In conclusion, synthesis of (*E*)(2,2-(1,3-diphenyl-1*H*-pyrazole-4-yl)methylene)hydrazinyl)-2-oxoethyl)benzamide derivatives from hippuric acid as the substrate has been achieved. The structures of the synthesized compounds were characterized on the basis of IR, NMR (¹H and ¹³C) and mass spectral data. The compounds **8j** and **8l** exhibited good inhibitory potential against all the four bacterial and two fungal strains but compound **8l** was found to be most active and could be used as a lead compound for further studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- V. Kumar, K. Kaur, G.K. Gupta and A.K. Sharma, *Eur. J. Med. Chem.*, **69**, 735 (2013a); <https://doi.org/10.1016/j.ejmech.2013.08.053>.
- K. Kaur, V. Kumar, A.K. Sharma and G.K. Gupta, *Eur. J. Med. Chem.*, **77**, 121 (2014); <https://doi.org/10.1016/j.ejmech.2014.02.063>.
- G.S. Hassan, S.M. El-Messery, F.A.M. Al-Omary and H.I. El-Subbagh, *Bioorg. Med. Chem. Lett.*, **22**, 6318 (2012); <https://doi.org/10.1016/j.bmcl.2012.08.095>.
- V. Kumar, R. Aggarwal, P. Tyagi and S.P. Singh, *Eur. J. Med. Chem.*, **40**, 922 (2005); <https://doi.org/10.1016/j.ejmech.2005.03.021>.
- R. Aggarwal, V. Kumar, P. Tyagi and S.P. Singh, *Bioorg. Med. Chem.*, **14**, 1785 (2006); <https://doi.org/10.1016/j.bmc.2005.10.026>.
- R.M. Mohareb, N.N.E. El-Sayed and M.A. Abdelaziz, *Molecules*, **17**, 8449 (2012); <https://doi.org/10.3390/molecules17078449>.
- J.P. Raval, A.B. Shah, N.H. Patel, H.V. Patel, P.S. Patel, K.K. Bhatt and K.R. Desai, *Eur. J. Med. Chem.*, **2**, 238 (2011); <https://doi.org/10.5155/eurjchem.2.2.238-242.206>.
- G.K. Gupta, V. Kumar and V. Kumar, *Res. J. Chem. Environ.*, **15**, 90 (2011).
- V. Kumar, K. Kaur, G.K. Gupta, A.K. Gupta and S. Kumar, *Recent Pat. Inflamm. Allergy Drug Discov.*, **7**, 124 (2013); <https://doi.org/10.2174/1872213X11307020004>.
- M. Abdel-Aziz, G.E.-D.A. Abu-Rahma and A.A. Hassan, *Eur. J. Med. Chem.*, **44**, 3480 (2009); <https://doi.org/10.1016/j.ejmech.2009.01.032>.
- H. Bektas, N. Karaali, D. Sahin, A. Demirbas, S.A. Karaoglu and N. Demirbas, *Molecules*, **15**, 2427 (2010); <https://doi.org/10.3390/molecules15042427>.
- R. Kumar, R.R. Nair, S.S. Dhiman, J. Sharma and O. Prakash, *Eur. J. Med. Chem.*, **44**, 2260 (2009); <https://doi.org/10.1016/j.ejmech.2008.06.004>.
- M. Shiradkar, G.V. Suresh Kumar, V. Dasari, S. Tatikonda, K.C. Akula and R. Shah, *Eur. J. Med. Chem.*, **42**, 807 (2007); <https://doi.org/10.1016/j.ejmech.2006.12.001>.
- K. Sztanke, T. Tuzimski, J. Rzymowska, K. Pasternak and M. Kandefer-Szerszeń, *Eur. J. Med. Chem.*, **43**, 404 (2008); <https://doi.org/10.1016/j.ejmech.2007.03.033>.
- L.T. Yu, M.T. Ho, C.Y. Chang and T.K. Yang, *Tetrahedron Asym.*, **18**, 949 (2007); <https://doi.org/10.1016/j.tetasy.2007.03.015>.
- B.D. Mistry, K.R. Desai, J.A. Patel and N.I. Patel, *Indian J. Chem.*, **51B**, 746 (2012).
- M.D. Amdare, K.R. Jogdand, L. Kathane, N. Ganesh, C.D. Padole, and D.K. Mahapatra D, *J. Pharm. Chem. Biol. Sci.*, **5**, 216 (2017).
- C.A.B. Rodrigues, J.M.G. Martinho and C.A.M. Afonso, *J. Chem. Educ.*, **92**, 1543 (2015); <https://doi.org/10.1021/ed500212t>.
- C. Nagamani, S.M. Basavarajiah, S. Nagaraj and C.S. Devi, *Int. J. Comput. Eng. Res.*, **6**, 2250 (2016).
- R. Ahmed, *Indian J. Soc. Res.*, **13**, 126 (2017).