Antibacterial Evaluation of Some Synthetic 3,4-Dihydroisocoumarins

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SUMMARY. Synthesis of some 3,4-dihydroisocoumarins related to well known bioactive natural dihydroiso-
cumarins viz. scorzocreticin, annulatomarin and montroumarin, have been carried out starting from 3,5-
dimethoxy-4-methylphenyl acetic acid. Homophthalic acid was condensed with various acid chlorides (a-e) to af-
ford the corresponding 6,8-dimethoxy-7-methyl-3-arylisocoumarins (2a-e). The alkaline hydrolysis of iso-
cumarins yields keto-acids (3a-e), which were then reduced to hydroxyacids, followed by cyclodehydration
with acetic anhydride furnish corresponding 3,4-dihydroisocoumarins (4a-e). Finally, demethylation of 3,4-dihy-
droisocoumarins was carried out to afford 6,8-dihydroxy-7-methyl-3-aryl-3,4-dihydroisocoumarins (5a-e). In víti-
ro antibacterial screening of all the synthesized compounds were carried out against ten bacterial strains, and
they display moderate activity towards various Gram negative and Gram positive bacteria, as compared to the
standard drugs.

INTRODUCTION

Naturally occurring lactones isocoumarins and 3,4-dihydroisocoumarins are secondary
metabolites, numerous exists in nature variety of species like fungi, bacteria, lichens, molds
and insects and also present among various in-
sict pheromones and venoms 1-5. Such metabo-
lites were also extracted from higher plants to
lesser extent and rich sources were found in
marine organisms 4-10.

These secondary metabolites exhibit a vari-
ety of important and interesting biological 11-15
activities like immunomodulatory, antiallergic,
anti-inflammatory, antifungal, antimicrobial,
necrotic, antidiabetic, antibacterial, antitumor,
cytotoxic, antitubercular and they also posses
extensive range of remarkable pharmaceutical
properties 14-16 includes antiangiogenic, differen-
tiation including activity against leukemic cells
17-22 and anticalmodulin-sensitive cyclic guano-
sine 3’,5’-monophosphate phosphodiesterase
effects etc.

MATERIAL AND METHODS

General

1H NMR were recorder on a Brucker AM-300
spectrophotometer and chemical shifts of 1H
NMR are reported in parts per million (ppm).
The melting point was determined on Stautrt
SMP3 melting point apparatus and is uncorrect-
ed. FT IR spectra were recorded using Shimadzu IR 460 spectrophotometer by Attenuated Total Reflectance (ATR) method. The elemental analysis was performed on leco CHNS-932 analyzer.

**Synthesis of 6,8-dimethoxy-7-methyl-(3-aryl)isocoumarins (2a-e)**

A solution of 3,5-dimethoxy-4-methyl homophthalic acid (1) (2.0 mmol) and aromatic acid chlorides (3.1 mmol) ((a-e) were heated under reflux at an internal temperature of 200 °C for 3-4 hr. The reaction mixture was concentrated, then extracted with ethyl acetate and then dried over anhydrous (Na2SO4). Isocoumarins (2a-e) were then purified by preparative thin layer chromatography using petroleum ether and ethyl acetate, (7:3) as eluent.

**6,8-Dimethoxy-7-methyl-3-(phenyl)isocoumarin (2a)**

Yield = 86%; Rf = 0.6; M.P. = 108-110 °C; IR (KBr) νmax: 3033 (C=C-H), 2936 (C-H), 1716 (C=O), 1583 (C=C) cm –1; 1H NMR (CDCl3, δ ppm): 7.97-8.13 (5H, m, Ph), 7.86 (1H, s, H-5), 7.69 (2H, d, J = 7.1 Hz, H-2',H-6'), 7.71 (2H, d, J = 7.1 Hz, H-3',H-5'), 7.41 (1H, s, H-5), 6.72 (1H, s, H-4), 3.74 (3H, s, Ar-CH3); 13C NMR (CDCl3, δ ppm): 164.7 (C=O), 146.1 (C-3), 137.6 (C-4'), 135.3 (C-10), 135.6 (C-4'), 130.2 (C-1'), 127.8 (C-3',C-5'), 126.5 (C-4'), 124.7 (C-2',C-6'), 118.6 (C-7), 113.3 (C-4), 108.5 (C-5), 62.6 (-OCH3), 62.4 (-OCH3), 28.6 (Ar-CH3); MS (70 eV): m/z (%); [M+.] 326 (56), 313 (27), 232.5 (48), 219 (43), 191 (100 %), 135 (58), 107 (36), 77 (21) ; Anal. Calcd for C19H16O5: C, 69.94; H, 4.87. Found: C, 69.67; H, 4.53.

**3-(4-Chlorophenyl)-6,8-dimethoxy-7-methylisocoumarin (2c)**

Yield = 82%; Rf = 0.55; M.P. = 153-155 °C; IR (KBr) νmax: 3037 (C=C-H), 1724 (C=O), 1574 (C=C) cm –1; 1H NMR (CDCl3, δ ppm): 7.97 (1H, d, J = 7.6 Hz, H-3',H-5'), 7.76 (1H, s, H-5), 6.85 (1H, s, H-4), 3.87 (3H, s, -OCH3), 3.82 (3H, s, -OCH3), 3.67 (3H, s, -OCH3); 13C NMR (CDCl3, δ ppm): 161.4 (C=O), 142.5 (C-3), 138.7 (C-6), 137.3 (C-8), 136.5 (C-4'), 135.6 (C-10), 133.5 (C-9), 130.6 (C-1'), 127.4 (C-3',C-5'), 124.6 (C-2',C-6'), 122.5 (C-7), 119.2 (C-4), 112.3 (C-5), 56.6 (-OCH3), 55.3 (-OCH3), 53.7 (4'-OCH3), 28.2 (Ar-CH3); MS (70 eV): m/z (%); [M+1] 326 (51), 219 (43), 191 (100 %), 135 (58), 107 (36), 77 (21) ; Anal. Calcd for C19H16ClO5: C, 64.94; H, 4.52. Found: C, 64.87; H, 4.36.

**Synthesis of 2,4-dimethoxy-3-methyl-6-(2-oxoaryl)benzoic acids (3a-e)**

A stirred solution of 6,8-dimethoxy-7-methyl-3-aryl-isocoumarins (2a-e) (1.42 mmol) in ethanol (20 mL) was treated with 5% KOH (40 mL) and the mixture refluxed for 4 h. After cool-
ing the reaction mixture, most of the ethanol was evaporated under reduced pressure. Cold water (20 mL) was added and the mixture acidified with dilute hydrochloric acid when solid was precipitated. Filtration followed by drying under vacuum afforded keto-acid derivatives (3a-e).

2.4-Dimethoxy-3-methyl-6-(2-oxo-2-phenylethyl)benzoic acid (3a)

Yield = 87 %; Rf = 0.4; M.P. = 168-170 °C; IR (KBr) νmax: 3324 (O-H), 3034 (C=C-H), 1738 (carboxylic C=O), 1714 (ketonic C=O), 1587 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 11.4 (1H, s, -COOH), 8.02 (2H, d, J = 7.4 Hz, H-2',H-6'), 7.87 (1H, s, H-5), 7.76 (1H, dd, J = 7.2,7.1 Hz, H-4'), 7.62 (2H, dd, J = 7.3,7.1 Hz, H-3',H-5'), 4.33 (2H, s, -CH₂), 3.92 (3H, s, -OCH₃), 3.88 (3H, s, -OCH₃), 2.61 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 194.4 (ketonic C=O), 166.8 (carboxylic C=O), 142.5 (C-1), 138.6 (C-2), 137.5 (C-1'), 136.8 (C-6), 132.5 (C-4), 126.4 (C-2',C-6'), 124.6 (C-3',C-5'), 122.4 (C-4'), 121.7 (C-3), 115.4 (C-5), 62.6 (-OCH₃), 58.3 (-OCH₃), 44.3 (C-1'), 28.5 (Ar-CH₃); MS (70 eV): m/z (%); [M⁺] 314 (42), 296 (55), 270 (68), 237 (31), 219 (100 %), 192 (24), 161 (16), 105 (27); Anal. calcd for C₁₈H₁₇O₅Cl: C, 60.04 H, 4.76; Found: C, 59.70 H, 5.02.

2.4-Dimethoxy-6-[2-(4-methoxyphenyl)-2-oxoethyl]-3-methylbenzoic acid (3b)

Yield = 84 %; Rf = 0.35; M.P. = 168-169 °C; IR (KBr) νmax: 3326 (O-H), 3041 (C=C-H), 1736 (carboxylic C=O), 1712 (ketonic C=O), 1567 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 10.7 (1H, s, -COOH), 8.07 (1H, s, H-5), 7.91 (2H, d, J = 7.4 Hz, H-2',H-6'), 7.6 (2H, d, J = 7.2 Hz, H-3',H-5'), 4.27 (2H, s, -CH₂), 3.78 (3H, s, -OCH₃), 3.74 (3H, s, -OCH₃), 2.72 (3H, s, Ar-CH₃), 2.32 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 195.2 (ketonic C=O), 166.4 (carboxylic C=O), 141.6 (C-1), 138.5 (C-2), 136.2 (C-4), 135.2 (C-1'), 132.4 (C-2',C-6'), 128.6 (C-3',C-5'), 126.5 (C-4'), 122.3 (C-3), 116.7 (C-5), 62.3 (-OCH₃), 61.8 (-OCH₃), 44.5 (C-1'), 28.6 (Ar-CH₃), 27.4 (Ar-CH₃); MS (70 eV): m/z (%); [M⁺] 314 (53), 284 (60), 237 (26), 219 (100 %), 192 (27), 119 (32), 101 (23); Anal. calcd for C₁₉H₁₇O₇: C, 64.99 H, 6.14; Found: C, 64.33 H, 5.97.

2.4-Dimethoxy-6-[2-(4-nitrophenyl)-2-oxoethyl]-3-methylbenzoic acid (3c)

Yield = 82 %; Rf = 0.45; M.P. = 157-158 oC; IR (KBr) νmax: 3411 (O-H), 3053 (C=C-H), 1738 (carboxylic C=O), 1715 (ketonic C=O), 1574 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 11.3 (1H, s, -COOH), 8.11 (2H, d, J = 7.4 Hz, H-2',H-6'), 7.97 (2H, d, J = 7.2 Hz, H-3',H-5'), 7.47 (1H, s, Ar-CH₃), 4.28 (2H, s, -CH₂), 3.91 (3H, s, -OCH₃), 3.86 (3H, s, -OCH₃), 2.68 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 197.6 (ketonic C=O), 167.4 (carboxylic C=O), 143.5 (C-1), 138.2 (C-2), 137.3 (C-1'), 136.2 (C-4), 134.5 (C-2',C-6'), 134.3 (C-4'), 132.8 (C-3',C-5'), 127.5 (C-3), 116.4 (C-5), 62.6 (-OCH₃), 62.2 (-OCH₃), 44.7 (C-1'), 30.3 (Ar-CH₃); MS (70 eV): m/z (%); [M⁺] 348 (40), 350.5 (M+2)+ (32), 330.5 (65), 304.5 (37), 237 (23), 219 (100 %), 192 (22), 138.5 (17), 111.5 (20); Anal. calcd for C₁₉H₁₇O₄Cl: C, 61.97 H, 4.86; Found: C, 61.86 H, 4.68.
Synthesis of 6,8-Dimethoxy-7-methyl-3-(aryl)-3,4-dihydroisocoumarin (4a-e)

Sodium borohydride (18 mmol) was added portion wise to a stirred solution of keto acids (3a-e) (0.66 mmol) in ethanol (25 mL) and water (75 mL). The reaction mixture was stirred for 2 hr at room temperature, diluted with water (150 mL), acidified with conc. HCl and stirred for further 2 hr. It was then saturated with ammonium sulfate, and extracted with ethyl acetate (3 x 10 mL). The organic layer dried over anhydrous magnesium sulfate and concentrated. 6,8-Dimethoxy-7-methyl-3-aryl-3,4-dihydroisocoumarins (4a-e) were purified by preparative thin layer chromatography using (petroleum ether and ethyl acetate 7:3) as eluent.

6,8-Dimethoxy-7-methyl-3,4-dihydroisocoumarin (4a)

Yield = 86%; Rf = 0.5; M.P. = 93-94 °C; IR (KBr) νmax: 3042 (C=C-H), 2937 (C-H), 1716 (C=O), 1585 (C=C cm-1; 1H NMR (CDCl3, δ ppm): 7.87 (2H, d, J = 7.2 Hz, H-2',H-6'), 7.74 (2H, dd, J = 7.1,6.8 Hz, H-3',H-5'), 7.65 (1H, m, H-4'), 6.86 (1H, s, Ar-H-5), 5.43 (1H, dd, Jtrans = 12.2 Hz, Jcis = 3.4 Hz, H-3), 3.85 (3H, s, -OCH3), 3.78 (3H, s, -OCH3), 3.32 (1H, dd, Jgem = 16.1 Hz, Jcis = 12.2 Hz, H-4), 3.17 (1H, dd, Jgem = 12.4 Hz, Jcis = 3.6 Hz, H-4'), 2.84 (3H, s, Ar-CH3); 13C NMR (CDCl3, δ ppm): 167.3 (C=O), 142.3 (C-6), 141.6 (C-8), 139.5 (C-10), 138.1 (C-1'), 137.2 (C-9), 132.8 (C-7), 131.7 (C-5), 130.6 (C-2',C-6'), 124.5 (C-3',C-5'), 118.4 (C-4'), 82.6 (C-3), 57.2 (6-OCH3), 56.6 (8-OCH3), 43.2 (C-4); MS (70 eV): m/z: [M+H]+ 343 (44), 297 (16), 192 (100 %), 164 (41), 137 (22), 105 (62), 77 (54); Anal. calcd for C19H20O4: C, 73.04 H, 6.44; Found: C, 72.97 H, 6.87.

6,8-Dimethoxy-7-methyl-3-(4-methylphenyl)-3,4-dihydroisocoumarin (4b)

Yield = 84%; Rf = 0.5; M.P. = 109-110 °C; IR (KBr) νmax: 3028 (C=C-H), 2934 (C-H), 1724 (C=O), 1575 (C=C cm-1; 1H NMR (CDCl3, δ ppm): 7.81 (2H, d, J = 6.8 Hz, H-2',H-6'), 7.73 (2H, dd, J = 7.2 Hz, H-3',H-5'), 6.68 (1H, s, H-5), 5.34 (1H, dd, Jtrans = 12.4 Hz, Jcis = 3.7 Hz, H-3), 3.83 (3H, s, 6-OCH3), 3.76 (3H, s, 8-OCH3), 3.34 (1H, dd, Jgem = 16.2 Hz, Jtrans = 12.4 Hz, H-4), 3.15 (1H, dd, Jgem = 12.4 Hz, Jcis = 3.7 Hz, H-4), 2.81 (3H, s, Ar-CH3); 13C NMR (CDCl3, δ ppm): 166.3 (C=O), 141.5 (C-6), 140.7 (C-8), 139.2 (C-10), 138.4 (C-1'), 136.9 (C-3), 133.6 (C-4'), 132.7 (C-2',C-6'), 131.2 (C-5), 130.5 (C-3',C-5'), 127.4 (C-7), 81.5 (C-3'), 56.7 (-OCH3), 56.2 (-OCH3), 42.6 (C-4'), 27.3 (Ar-CH3), 23.7 (4'-CH3); MS (70 eV): m/z (%); [M+] 312 (56), 192 (100 %), 164 (44), 137 (63), 107 (28); Anal. calcd for C10H12O4: C, 73.04 H, 6.44; Found: C, 72.93 H, 6.26.
3.92 (3H, s, -OCH₃), 3.87 (3H, s, -OCH₃), 3.37 (1H, dd, $J_{gem} = 16.2$ Hz, $J_{trans} = 12.2$ Hz, H-4), 3.16 (1H, dd, $J_{gem} = 12.4$ Hz, $I_{trans} = 3.6$ Hz, H-4), 2.65 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 164.6 (C-60), 143.5 (C-61), 142.7 (C-62), 141.2 (C-10), 139.4 (C-11), 137.8 (C-9), 136.7 (C-4), 133.5 (C-2, C-6), 132.3 (C-5), 131.1 (C-3, C-5'), 128.6 (C-7), 88.3 (C-3), 56.3 (-OCH₃), 55.8 (-OCH₃), 44.3 (C-4), 28.8 (Ar-CH₃); MS (70 eV): $m/z$ (%) ; [M⁺] 325.6 (61), 334.5 [M+2] (38), 192 (100%), 164 (33), 139.5 (65), 111.5 (53); Anal. calc'd for C₁₈H₁₄O₄Cl: C, 64.94 H, 5.13; Found: C, 64.82 H, 5.07.

**Synthesis of 6,8-Dihydroxy-7-methyl-3-(aryl)-3,4-dihydroisocoumarins (5a-e)**

Dihydroisocoumarins (4a-e) (1.25 mmol) were dissolved in ethanethiol (3.5 mL) and solution was cooled on ice. Aluminium chloride (3.8 mmol) was added in 3 portions with an interval of 30 min and stirred it on ice for 1 h. The reaction was quenched with water, alkalinized (10% NaHCO₃) and extracted with ethyl acetate, washed once with brine, dried over sodium sulfate and concentrated to give 6,8-dihydroxy-7-methyl-3-aryl-3,4-dihydroisocoumarins (5a-e).

**6,8-Dihydroxy-7-methyl-3-phenyl-3,4-dihydroisocoumarin (5a)**

Yield = 84 %; RI = 0.4; M.P. = 124–125 °C; IR (KBr) $ν_{max}$: 3482 (O-H), 3031 (C=C-H), 1723 (C=O), 1581 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 7.95 (2H, d, $J = 6.8$ Hz, H-2, H-6), 7.73 (2H, dd, $J = 6.7$, 5.8 Hz, H-3, H-5), 7.64 (1H, dd, $J = 6.4$, 5.6 Hz, H-5'), 6.87 (1H, s, H-5), 5.35 (1H, dd, $J_{trans} = 12.2$ Hz, $J_{cis} = 3.8$ Hz, H-3), 4.67 (2H, s, -OH), 3.38 (1H, dd, $J_{gem} = 16.4$ Hz, $J_{trans} = 12.2$ Hz, H-4), 3.16 (1H, dd, $J_{gem} = 12.2$ Hz, $I_{cis} = 3.7$ Hz, H-4), 2.64 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 166.8 (C-60), 148.5 (C-61), 147.2 (C-62), 142.5 (C-10), 138.4 (C-11), 137.5 (C-9), 132.3 (C-7), 128.5 (C-2, C-6), 126.2 (C-3, C-5), 122.7 (C-5), 118.6 (C-4), 82.4 (C-3), 44.4 (C-4), 29.5 (Ar-CH₃); MS (70 eV): $m/z$ (%) ; [M⁺] 270 (39), 164 (100%), 136 (48), 105 (56), 77 (31); Anal. calc'd for C₁₇H₁₄O₄: C, 71.12 H, 5.17; Found: C, 71.02 H, 5.09.

**6,8-Dihydroxy-7-methyl-3-(4-chlorophenyl)-3,4-dihydroisocoumarin (5c)**

Yield = 81 %; RI = 0.25; M.P. = 137–138 °C; IR (KBr) $ν_{max}$: 3482 (O-H), 3037 (C=C-H), 1723 (C=O), 1778 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 8.03 (1H, d, $J = 7.1$ Hz, H-3, H-4), 7.95 (1H, d, $J = 6.7$ Hz, H-2, H-6), 6.96 (1H, s, H-5), 5.48 (1H, dd, $J_{trans} = 12.1$ Hz, $I_{cis} = 3.5$ Hz, H-3), 4.87 (2H, s, -OH), 3.51 (1H, dd, $J_{gem} = 16.2$ Hz, $J_{trans} = 12.1$ Hz, H-4), 3.32 (1H, dd, $J_{gem} = 12.1$ Hz, $J_{cis} = 3.6$ Hz, H-4), 2.87 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 167.5 (C-50), 148.7 (C-60), 147.6 (C-61), 143.8 (C-10), 142.4 (C-11), 139.2 (C-9), 138.5 (C-4), 134.6 (C-2, C-6), 133.4 (C-5), 122.7 (C-3, C-5), 129.3 (C-3, C-7), 83.8 (C-3), 44.8 (C-4), 29.8 (Ar-CH₃); MS (70 eV): $m/z$ (%) ; [M⁺] 315 (38), 287 (35), 269 (27), 164 (100%), 136 (26), 105 (32), 91 (14); Anal. calc'd for C₁₇H₁₆ClO₄: N, 6.95; H, 4.15; N, 4.43; Found: C, 60.87 H, 4.11, N, 4.36.

**6,8-Dihydroxy-7-methyl-3-(4-nitrophenyl)-3,4-dihydroisocoumarin (5e)**

Yield = 81 %; RI = 0.25; M.P. = 161–162 °C; IR (KBr) $ν_{max}$: 3461 (O-H), 3042 (C=C-H), 1726 (C=O), 1582 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 7.85 (2H, d, $J = 7.3$ Hz, H-3, H-5), 7.68 (2H, d, $J = 7.1$ Hz, H-2, H-6), 6.92 (1H, s, H-5), 5.32 (1H, dd, $J_{trans} = 12.4$ Hz, $J_{cis} = 3.7$ Hz, H-3), 4.82 (2H, s, -OH), 3.42 (1H, dd, $J_{gem} = 16.2$ Hz, $J_{trans} = 12.1$ Hz, H-4), 3.25 (1H, dd, $J_{gem} = 12.1$ Hz, $J_{cis} = 3.8$ Hz, H-4), 2.84 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 166.3 (C-60), 148.2 (C-61), 147.5 (C-10), 142.4 (C-11), 140.3 (C-12), 136.7 (C-9), 133.4 (C-4), 131.6 (C-2, C-6), 131.7 (C-5), 130.3 (C-3, C-5), 128.5 (C-7), 83.4 (C-3), 56.5 (4-OCH₃), 44.2 (C-4), 29.5 (Ar-CH₃); MS (70 eV): $m/z$ (%) ; [M⁺] 300 (40), 164 (100%), 136 (27), 135 (48), 107 (23); Anal. calc'd for C₁₇H₁₆NO₄: C, 68.02 H, 5.34; Found: C, 67.88 H, 5.22.

**6,8-Dihydroxy-7-methyl-3-(4-chlorophenyl)-3,4-dihydroisocoumarin (5e)**

Yield = 87 %; RI = 0.35; M.P. = 126–127 °C; IR (KBr) $ν_{max}$: 3472 (O-H), 3036 (C=C-H), 1721
RAFIQUE H., SAEED A. & MUMTAZ A. (C=O), 1775 (C=C) cm⁻¹; ¹H NMR (CDCl₃, δ ppm): 7.93 (1H, d, J = 7.1 Hz, H-3',H-5'), 7.88 (1H, d, J = 6.8 Hz, H-2',H-6'), 6.93 (1H, s, H-5), 5.41 (1H, dd, Jtrans = 12.1 Hz, Jcis = 5.6 Hz, H-3), 4.85 (2H, s, -OH), 3.46 (1H, dd, Jgem = 16.1 Hz, Jtrans = 12.2 Hz, H-3), 3.28 (1H, dd, Jgem = 12.2 Hz, Jcis = 3.7 Hz, H-3'), 2.85 (3H, s, Ar-CH₃); ¹³C NMR (CDCl₃, δ ppm): 165.2 (C=O), 148.5 (C-6), 147.4 (C-8), 143.5 (C-10), 141.5 (C-1'), 138.3 (C-9), 137.7 (C-4'), 133.5 (C-2',C-6'), 132.1 (C-5), 131.3 (C-3',C-5'), 128.7 (C-7), 83.5 (C-3), 44.6 (C-4), 29.6 (Ar-CH₃); MS (70 eV): m/z (%); [M + .] 304.5 (38), 306.5 [(M+2) + .] (23), 164 (100 %), 136.5 (21), 139.5 ([M+2]+) (23), 164 (100 %), 136.5 (21), 139.5 (53), 111.5 (48); Anal. calcd for C₁₆H₁₃O₄Cl: C, 63.04 H, 4.26; Found: C, 62.95 H, 4.14.

RESULTS AND DISCUSSION
Various aromatic carboxylic acids were converted into their corresponding acid chlorides, which were condensed with 3,5-dimethoxy-4-methylhomopthalic acid at 200 °C to afford 3-aryl-isocoumarins (Fig. 1).

In IR spectra, the lactonic carbonyl absorptions peaks of isocoumarins appeared at 1716-1726 cm⁻¹. In ¹H NMR they showed characteristic singlets for H-4 protons at δ 6.52-6.85, respectively and in ¹³C NMR spectra characteristic lactonic carbonyl carbon peaks observed at δ 162.4-171.5 ppm. The mass spectra of the isocoumarins (2a-e) showed base peaks observed at m/z 191.

Alkaline hydrolysis of isocoumarins (2a-e) furnishes keto-acid derivatives (3a-e). The keto-acids were reduced to corresponding hydroxy acids by sodium borohydride, followed by cyclodehydration with acetic anhydride yields 3,4-dihydroisocoumarins (4a-e). In IR spectra, the lactonic carbonyl absorptions peaks of dihydroisocoumarins appeared at 1716-1726 cm⁻¹. In ¹H NMR the H-4 protons showed characteristic two double doublets ranges from δ 3.32-3.37 ppm. The H-3 proton of 3-aryl substituted 3,4-dihydroisocoumarins gives double doublet at δ 5.31-5.46 ppm. In ¹³C NMR spectra characteristic lactonic carbonyl carbon peaks observed at δ 164.6-167.6 ppm. The mass spectra of the dihydroisocoumarins (4a-e) showed base peaks observed at m/z 192.

In IR spectra, the characteristic -NH peaks observed in the range of 3456-3484 cm⁻¹ and lactonic carbonyl absorptions peaks of dihydroxy derivatives (5a-e) appeared at 1721-1726 cm⁻¹. In ¹H NMR the characteristic broad singlets for -OH protons appeared at δ 4.67-4.87 ppm. The H-4 protons showed two characteristic double doublets ranges from δ 3.38-3.51 ppm. The H-3 proton of 3-aryl substituted dihydroxy derivatives gives double doublet at δ 5.32-5.48 ppm. In ¹³C NMR spectra characteristic lactonic carbonyl carbon peaks observed at δ 165.2-167.6 ppm. The mass spectra of the dihydroxy derivatives (5a-e) showed base peaks observed at m/z 164.

Antibacterial assay
In vitro evaluation of antibacterial activity of the isocoumarins (2a-e), 3,4-dihydroisocoumarins (4a-e) and their 6,8-dihydroxy derivatives (5a-e), were carried out by agar well diffusion assay technique against ten different Gram positive and Gram negative bacterial strains. An-

![Figure 1: Synthesis of 6,8-Dihydroxy-7-methyl-3-(aryl)-3,4-dihydroisocoumarins (5a-e)](image-url)
tibacterial activity was determined by using the Mueller Hinton Agar (MHA). The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5 Mc-Farland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6 mm sized borer to make the wells. The sample dilutions were prepared by dissolving each sample (1.0 mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0 mg/ml), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 h. Antibacterial activity of all the synthesized compounds were determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control. The experiments were repeated thrice to minimize the errors, only the mean values are reported.

Antibacterial evaluation of 3-arylated isocoumarin derivatives (2a-e), the compounds (2d) and (2e) are more active against various bacterial strains. The antibacterial activity results of 3-arylated isocoumarin is presented in Table 1. Among 3,4-dihydroisocoumarins (4a-e) and 6,8-dihydroxy-3,4-dihydroisocoumarin derivatives (5a-e), the compounds (4d) and (5e) show good antibacterial activities. The antibacterial activity results of 3-arylated-3,4-dihydroisocoumarins and 6,8-dihydroxy-3,4-dihydroisocoumarins is presented in Tables 2 and 3.

CONCLUSION

Some 3,4-hydroxyisocoumarins and analogues of scorzocretin, annulatomarin and montroumarin, have been carried out successfully synthesized by adopting multistep synthetic strategy. Biological screening of all the synthesized compounds were carried out against ten bacterial strains, it was concluded that isocoumarins (2a-e) and 3,4-dihydroisocoumarins (4a-e) are more active than 6,8-dihydroxy-3,4-dihydroisocoumarin derivatives (5a-e).

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Table 1. Antibacterial Bioassay of Isocoumarins (2a-e).

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Table 2. Antibacterial Activity of 3,4-Dihydroisocoumarins (4a-e).
Acknowledgement. The authors are grateful to the Pakistan Science Foundation (PSF) for a research grant under Project No. PSF/Res/C-QU/Chem (395).

REFERENCES


Table 3. Antibacterial activity of 6,8-Dihydroxy-3,4-dihydroisocoumarins (5a-e).