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Bis-(1*H*-2-benzopyran-1-one) derivatives: Synthesis and antimicrobial evaluation

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Abstract. The aim of the present study was to synthesize isocoumarin heterocycles and to elucidate the potential role of these compounds as biological active agents. A new series of isocoumarin derivatives containing two six-membered lactone rings is reported. 3-Aroyl-substituted isocoumarins (**3**) obtained by condensing 2-carboxy benzaldehyde (**1**) with bromoacetophenone derivatives (**2**) was further reacted with different aromatic aldehydes (**4**) affording bis-(1*H*-2-benzopyran-1-one) derivatives (**5**). This short review compiles examples of most promising antibacterial, antifungal and analgesic bis-(1H-2-benzopyran-1-one) derivatives. The products were characterized on the basis of analytical and spectral (IR, ^{1}HNMR, C^{13}NMR, Mass) data. The biological activity study revealed that all compounds showed promising activities and bis-(1*H*-2-benzopyran-1-one) derivatives (**5**) were found to be more active than 3-aroyl-substituted isocoumarins (**3**).

Keywords. 2-Carboxy benzaldehyde; 3-aroyl isocoumarin; bis-(1*H*-2-benzopyran-1-one) derivatives; antibacterial activity; antifungal activity; analgesic activity.

1. Introduction

Among the 877 new molecule entities worldwide introduced as drugs during 1981–2002, 61% can be traced to or were inspired by natural products.[1] These include natural products, natural product derivatives, synthetic compounds with natural product derived pharmacophores and synthetic compounds designed on the basis of knowledge gained from a natural product.[2] Similarly, isocoumarins are an important class of naturally occurring biologically active lactones, originating from a variety of natural sources, with multiple biological activities.

Several articles dealing with the varied physiological activities of isocoumarin derivatives have been published, describing their anti-allergic, antimicrobial, immunomodulatory, cytotoxic, antifungal, antiinflammatory and anti-angiogenic action. For the past many years, the study of biological activities of isocoumarin derivatives has been the aim of many researchers.[3–11]

In our earlier studies,[12–14] we found an increase in the bacteriological properties of isocoumarins on introducing various substituted alkyl/acyl, aroyl and aminomethyl linkages at different positions in isocoumarin nucleus. Also, the structure activity relationships of isocoumarins have revealed that the mere presence of isocoumarin moiety in any compound itself is an essential feature of their pharmacological action.[15]

Furthermore, in recent decades, an increased incidence of microbial infection has been observed as a consequence of the growing number of immunocompromised patients and the frequent use of antibacterial and cytotoxic drugs.

Despite the fact that the isocoumarin skeleton is found in a variety of natural products, the dimerization of isocoumarin itself has not been investigated. There exist only few relevant examples in literature,[16–18] related to the dimerization of coumarins and its different derivatives along with their biological applications.

Based on the wide spectrum of biological profile of isocoumarins, and their increasing importance in pharmaceutical and biological fields, and in continuation of our ongoing research on biologically active heterocycles, it was thought to be interest to accommodate two identical isocoumarin moieties in a single molecular framework to synthesize some new heterocyclic compounds with potential biological activity.

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2. Experimental

2.1 *Materials and methods*

Reagents and solvents used in this study were of analytical grade and used without further purification. Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by Thin Layer Chromatography (TLC) on silica gel GF254 plates using UV/iodine as visualizing agent and Merk's silica gel (60–120 mesh) was used for column chromatographic purification. Infrared spectra were recorded on Fourier Transform Infra Red (FTIR) Perkin–Elmer spectrophotometer using potassium bromide optics. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer (400 MHz) using Tetramethylsilane (TMS) as internal standard and chemical shifts are given in ppm. Mass spectra were obtained using Thermo Scientific Corporation, DSQ II Mass Spectrometer. Elemental analyses were carried out on Perkin–Elmer C, H, N, S Analyser (Model-2400). Bromo acetophenone derivatives were prepared by literature method.[19] Antibacterial activity of newly synthesized compounds was tested *in vitro* in bacterial strains, *Staphylococcus aureus* (clinical strain) and *Escherichia coli* (DH5 alpha) using serial agar dilution (cup plate method)[20] and Dimethylformamide (DMF) were used as positive controls. Antifungal activity was performed *in vitro* against fungal strains *Fusarium pallidoroseum* and *Colletotrichum capsici*, using Potato Dextrose Agar Medium (Poisoned Food Technique).[21] Analgesic activity of the compounds was determined *in vivo* by tail flick method.[22] Gum acacia (2%) as control and analgin drug as standard were used.

2.2 *General method for synthesis of 3-aroyl -1H-2-benzopyran-1-one (**3a–3f**)*

2-Carboxy benzaldehyde (1 g, 0.006 mol) **1**, 2-bromo-1-(4-bromo-phenyl)-ethanone (1.85 g, 0.006 mol) **2**, K_2CO_3 (2.00 g, 0.012 mol) and ethyl methyl ketone (10 ml) were taken in a round bottom flask and refluxed for 10–12 h. Solvent was then removed, water added and extracted with ethyl acetate. Solvent layer was first washed with sodium bicarbonate and then with water and dried over anh. Na_2SO_4. After removal of solvent, the crude product was purified by column chromatography using petroleum ether (60–80°C)-ethyl acetate.

2.2a *3-(4′-Bromo-benzoyl)-1H-2-benzopyran-1-one (**3a**)*: White crystals, mp: 171°C; 76.0% yield; IR (KBr, υ, cm^{-1}): 1735 (γ lactone), 1600 (C=O), 680 (C-Br); Anal. Calcd $C_{16}H_9O_3Br$ (328.9 g): C, 58.37; H, 2.73; Found: C, 58.42; H, 3.06; ^{1}H NMR ($CDCl_3$)δ 7.42 (s, 1H, CH), 7.63–7.91 (m, 7H, aromatic protons), 8.44 (dd, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 164 (C=O, lactone), 142 (=C-O), 129 (C-Br), 132.3 (CH), 131.9 (CH), 136 (C), 115.6 (C), 136.5 (C), 128 (C), 130 (CH), 127.8 (CH), 133.6 (CH), 126 (CH); ms: m/z: 328.97 (M$^+$, 35%), 249(10), 174(19) and 146(100).

2.2b *3-(4′-Hydroxy-benzoyl)-1H-2-benzopyran-1-one (**3b**)*: White crystals, mp: 180°C; 64.2% yield; IR (KBr, υ, cm^{-1}): 1730 (γ lactone), 1610 (C=O), 3528 (OH); Anal. Calcd $C_{16}H_{10}O_4$ (266.0 g): C, 72.18; H, 3.75; Found: C, 72.00; H, 3.18; ^{1}H NMR ($CDCl_3$)δ 5.57 (s, 1H, OH), 7.61 (s, 1H, CH), 7.04–7.86 (m, 7H, aromatic protons), 8.34 (d, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 163 (C=O, lactone), 141.6 (=C-O), 163 (C-OH), 116 (CH), 131 (CH), 136 (C), 116 (C), 136.5 (C), 128 (C), 130 (CH), 127.8 (CH), 133.6 (CH), 126 (CH); ms: m/z: 266.04 (M$^+$, 48%), 185(10), 145(60) and 121(100).

2.2c *3-(2′, 4′-Dihydroxy-benzoyl)-1H-2-benzopyran-1-one (**3c**)*: Pinkish white crystals, mp: 116°C; 44.6% yield; IR (KBr, υ, cm^{-1}): 1733 (γ lactone), 1602 (C=O), 3556 (OH); Anal. Calcd $C_{16}H_{10}O_5$ (282.0 g): C, 68.08; H, 3.54; Found: C, 68.37; H, 4.08; ^{1}H NMR ($CDCl_3$)δ 5.51 (s, 1H, OH), 5.87 (s, 1H, OH), 7.59 (s, 1H, CH), 6.74–7.72 (m, 6H, aromatic protons), 8.29 (d, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 163 (C=O, lactone), 141 (=C-O), 165 (C-OH), 159 (C-OH), 108 (CH), 103 (CH), 132 (CH), 116.5 (C), 116 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH); ms: m/z: 282 (M$^+$, 26%), 264(8), 238(15), 173(47), 146(65), 137(60) and 57(83).

2.2d *3-(4′-Methoxy-benzoyl)-1H-2-benzopyran-1-one (**3d**)*: Yellow crystals, mp: 134°C; 62.8% yield; IR (KBr, υ, cm^{-1}): 1728 (γ lactone), 1556 (C=O), 1083 (OCH_3); Anal. Calcd $C_{17}H_{12}O_4$ (280.0 g): C, 72.85; H, 4.28; Found: C, 73.24; H, 3.94; ^{1}H NMR ($CDCl_3$)δ 3.95 (s, 3H, OCH_3), 7.42 (s, 1H, CH), 7.64–7.93 (m, 7H, aromatic protons), 8.43 (dd, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 163 (C=O, lactone), 141.7 (=C-O), 168 (C-OCH_3), 114.6 (CH), 131 (CH), 56 (CH_3), 129 (C), 116 (C), 136.5 (C), 128 (C), 130 (CH), 127.8 (CH), 133.6 (CH), 126 (CH); ms: m/z: 280 (M$^+$, 42), 252(54), 249(20), 145(74), 135(59) and 118(13).

2.2e *3-(Benzoyl)-1H-2-benzopyran-1-one (**3e**)*: White crystals, mp: 64°C; 65.1% yield; IR (KBr, υ, cm^{-1}): 1703 (γ lactone), 1629 (C=O); Anal. Calcd $C_{22}H_{14}O_3$ (326.0 g): C, 80.98; H, 4.29; Found: C, 81.29; H, 4.31;

^{1}H NMR ($CDCl_3$)δ 7.42 (s, 1H, CH), 7.33–7.90 (m, 12H, aromatic protons), 8.41 (dd, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 163 (C=O, lactone), 141.6 (=C-O), 134 (CH), 129 (CH), 130 (CH), 137 (C), 116 (C), 136.7 (C), 128.1 (C), 130 (CH), 127.6 (CH), 133 (CH), 126.1 (CH); ms: m/z: 325 (M^+-1, 45%), 300(62), 272(7), 221(21), 195(72), 174(27) and 146(95).

2.2f *3-(Dibenzofuran-3-carbonyl)-1H-2-benzopyran-1-one (**3f**)*: White crystals, mp: 110°C; 65.6% yield; IR (KBr, υ, cm^{-1}): 1720 (γ lactone), 1543 (C=O), 1253 (CO); Anal. Calcd $C_{22}H_{12}O_4$ (340.0 g): C, 77.64; H, 3.52; Found: C, 77.51; H, 4.01; ^{1}H NMR ($CDCl_3$)δ 7.76 (s, 1H, CH), 7.20–8.00 (m, 10H, aromatic protons), 8.45 (d, 1H, CH); ^{13}C NMR ($CDCl_3$)δ 187 (C=O, ketone), 163 (C=O, lactone), 141(=C-O), 132.8 (C), 113 (CH), 124.4 (CH), 122 (CH), 156 (C), 133.8 (C), 145 (C), 106.9 (C), 121 (CH), 123 (CH), 124 (CH), 111.8 (CH), 116 (C), 136.5 (C), 128 (C), 130 (CH), 127.8 (CH), 133.6 (CH), 126 (CH); ms: m/z: 340 (M^+, 12%), 312(31), 174(3), 168(10) and 146(82).

2.3 *General method for synthesis of bis-(1H-2-benzopyran-1-one) derivatives (**5a–5m**)*

A mixture of 3-aroyl isocoumarin **3b** (1 g, 0.0037 mol) was dissolved in ethanol and heated on water bath to get a clear solution. p-chlorobenzaldehyde **4** (0.264 g, 0.0018 mol) was added to this hot solution and refluxed for 18–20 h at 80°C. After the reaction was over, the solvent was distilled off and product recrystallized from ethanol.

2.3a *4, 4′- (4-Chlorobenzylidene)-bis-[3-(4′-hydroxy benzoyl)-1H-2-benzopyran-1-one] (**5a**)*: Pale white crystals, mp: 200°C; 74.9% yield; IR (KBr, υ, cm^{-1}): 1710 (γ lactone), 1495 (C=O), 3112 (OH); Anal. Calcd $C_{39}H_{23}O_8Cl$ (654.5 g): C, 71.61; H, 3.51; Found: C, 71.95; H, 3.14; ^{1}H NMR ($CDCl_3$)δ 5.75 (s, 1H, CH), 6.90–8.10 (m, 18H, aromatic protons), 8.39 (d, 2H, CH), 9.95 (s, 2H, OH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 186 (C=O, ketone), 134 (=C-O), 163 (C-OH), 39 (CH), 116 (CH), 131 (CH), 129 (C), 126 (C), 137 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 130.6 (C-Cl), 129 (CH), 130 (CH), 135.8 (C); ms: m/z: 480(7%), 410(5), 390(16), 375(24), 298(42), 266(50), 145(27), 121(49), 118(19) and 77(61).

2.3b *4, 4′-(Benzylidene)-bis-[3-(4′-hydroxy benzoyl)-1H-2-benzopyran-1-one] (**5b**)*: White crystals, mp: 197°C; 70.0% yield; IR (KBr, υ, cm^{-1}): 1718 (γ lactone), 1519 (C=O), 3252 (OH); Anal. Calcd $C_{39}H_{24}O_8$ (620.0 g): C, 75.48; H, 3.87; Found: C, 75.03; H, 4.17; ^{1}H NMR ($CDCl_3$)δ 3.65 (s, 2H, OH), 5.85 (s, 1H, CH), 6.98–7.99 (m, 19H, aromatic protons), 8.27 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 163.1 (C-OH), 38.7 (CH), 116 (CH), 131 (CH), 129.3 (C), 125.5 (C), 136.4 (C), 128.1 (C), 130 (CH), 127.3 (CH), 133.4 (CH), 126.1 (CH), 125.4(C-H), 128 (CH), 129 (CH), 137.8 (C); ms: m/z: 620 (M^+, 8%), 603(4), 577(26), 551(42), 509(8), 423(7), 368(38), 264(48) and 121(46).

2.3c *4, 4′-(4-Nitrobenzylidene)-bis-[3-(4′-hydroxy benzoyl)-1H-2-benzopyran-1-one] (**5c**)*: White crystals, mp: 198 °C; 68.2% yield; IR (KBr, υ, cm^{-1}): 1701(γ lactone), 1664 (C=O), 3428 (OH), 1486 (NO_2); Anal. Calcd $C_{39}H_{23}O_{10}N$ (665.0 g): C, 70.37; H, 3.45; N, 2.10; Found: C, 70.71; H, 3.92; N, 2.53; ^{1}H NMR ($CDCl_3$)δ 6.70 (s, 1H, CH), 7.54–8.19 (m, 18H, aromatic protons), 8.27 (d, 2H, CH) 9.35 (s, 2H, OH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 163.1 (C-OH), 38.5 (CH), 116.2 (CH), 131.1 (CH), 129.1 (C), 125.7 (C), 136.5 (C), 128 (C), 130 (CH), 127.3 (CH), 133.6 (CH), 126.1 (CH), 145.4(C-NO_2), 123 (CH), 130 (CH), 143.8 (C); ms: m/z: 663 (M^+-2, 2%), 619(8), 525(1), 479(2), 405(1), 266(30), 145(28) and 121(100).

2.3d *4, 4′-(4-Methoxybenzylidene)-bis-[3-(4′-hydroxy benzoyl)-1H-2-benzopyran-1-one] (**5d**)*: Yellow crystals, mp: 200°C; 72.0% yield; IR (KBr, υ, cm^{-1}): 1683 (γ lactone), 1624 (C=O), 3315 (OH), 1246 (OCH_3); Anal. Calcd $C_{40}H_{26}O_9$ (650.0 g): C, 73.84; H, 4.00; Found: C, 74.21; H, 4.38; ^{1}H NMR ($CDCl_3$)δ 3.84 (s, 3H, OCH_3), 7.41 (s, 1H, CH), 7.36–8.01 (m, 18H, aromatic protons), 8.35 (d, 2H, CH) 8.77 (s, 2H, OH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 163.1 (C-OH), 38.5 (CH), 116.2 (CH), 131.1 (CH), 129.1 (C), 125.7 (C), 136.5 (C), 128 (C), 130 (CH), 127.3 (CH), 133.6 (CH), 126.1 (CH), 159(O-CH_3), 56 (C – OCH_3), 114 (CH), 130 (CH), 130 (C); ms: m/z: 649 (M^+-1, 2%), 616 (1), 588 (3), 560 (5), 529(2), 406(9), 383(13), 329(10), 280(42), 145(24) and 135(84).

2.3e *4, 4′-(4-Nitrobenzylidene)-bis-[3-(4′-bromo benzoyl)-1H-2-benzopyran-1-one] (**5e**)*: Yellow crystals, mp: 144°C; 68.2% yield; IR (KBr, υ, cm^{-1}): 1720 (γ lactone), 1543 (C=O), 1446 (NO_2), 526 (C-Br); Anal. Calcd $C_{39}H_{21}O_8NBr_2$ (776.8 g): C, 59.18; H, 2.65; N, 1.77; Found: C, 58.97; H, 2.74; N, 2.05; ^{1}H NMR ($CDCl_3$)δ 7.50 (s, 1H, CH), 7.31–8.10 (m, 18H, aromatic protons), 8.44 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3

(=C-O), 129 (C-Br), 38.6 (CH), 132.2 (CH), 131.8 (CH), 135.7(C), 125.5 (C), 136.5 (C), 128.1 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 145 (C –NO_2), 123.3 (CH), 130 (CH), 144 (C); ms: m/z: 776.8 (M^+,1%), 617(1), 571(2), 540(12), 447.9(17), 435(3), 407(2), 328.9(48), 187(20), 183.9(51), 146(45), and 77(75).

2.3f *4, 4′-(4-Hydroxybenzylidene)-bis-[3-(4′-bromo benzoyl-1H-2-benzopyran-1-one] (**5f**)*: Yellow crystals, mp: 150°C; 69.0% yield; IR (KBr, υ, cm^{-1}): 1700 (γ lactone), 1643 (C=O), 3226 (OH), 580 (C-Br); Anal. Calcd $C_{39}H_{22}O_7Br_2$ (761.8 g): C, 61.43; H, 2.88; Found: C, 61.72; H, 3.12; ^{1}H NMR ($CDCl_3$)δ 6.03 (s, 1H, OH), 7.51 (s, 1H, CH), 7.30–8.15 (m, 18H, aromatic protons), 8.38 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 128.9 (C-Br), 33 (CH), 132.3 (CH), 131.9 (CH), 135 (C), 126 (C), 137 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.4 (CH), 126 (CH), 154 (C–OH), 116 (CH), 130.5 (CH), 131 (C); ms: m/z: 761.8 (M^+, 1%), 602(4), 585(6), 508(8), 432.9(15), 392(25), 183.9(10), 146(67) and 118(43).

2.3g *4, 4′-(Benzylidene)-bis-[3-(4′-bromo benzoyl)-1H-2-benzopyran-1-one] (**5g**)*: White crystals, mp: 176°C; 62.7% yield; IR (KBr, υ, cm^{-1}): 1689 (γ lactone), 1652 (C=O), 632 (C-Br); Anal. Calcd $C_{39}H_{22}O_6Br_2$ (745.8 g): C, 62.75; H, 2.94; Found: C, 63.18; H, 3.36; ^{1}H NMR ($CDCl_3$)δ 7.41 (s, 1H, CH), 7.44–8.15 (m, 18H, aromatic protons), 8.45 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163.2 (C=O, lactone), 186.8 (C=O, ketone), 134 (=C-O), 128.7 (C-Br), 32.6 (CH), 132 (CH), 131.9 (CH), 136 (C), 125.8 (C), 136.5 (C), 128.3 (C), 130 (CH), 128 (CH), 133.4 (CH), 126.1 (CH), 125.5(CH), 128.3 (CH), 129 (CH), 137.7 (C); ms: m/z: 745.8 (M^+, 4%), 665.9(7), 509(1), 481(29), 376(11), 483.9(5), 416.9(32), 328.9(31), 183.9(41) and 146(60).

2.3h *4, 4′-(4-Nitrobenzylidene)-bis-[3-(4′-methoxy benzoyl)-1H-2-benzopyran-1-one] (**5h**)*: Yellow crystals, mp: 128°C; 54.3% yield; IR (KBr, υ, cm^{-1}): 1710 (γ lactone), 1682 (C=O), 1065 (OCH_3), 1486 (NO_2); Anal. Calcd $C_{41}H_{27}O_{10}N$ (693.0 g): C, 70.99; H, 3.89; N, 2.02; Found: C, 71.23; H, 4.07; N, 1.94; ^{1}H NMR ($CDCl_3$)δ 3.74 (s, 6H, OCH_3), 7.55 (s, 1H, CH), 7.00–7.90 (m, 18H, aromatic protons), 8.20 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 168 (C-OCH_3), 56 (O–CH_3), 38.8 (CH), 115 (CH), 130.8 (CH), 129 (C), 125.5 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 145.5 (C –NO_2), 123.3 (CH), 130.1 (CH), 143.5 (C); ms: m/z: 693 (M^+, 16%), 662(10), 525(10), 479(13), 405(15), 280(36), 145(29) and 135(87).

2.3i *4, 4′-(4-Hydroxybenzylidene)-bis-[3-(4′-methoxy benzoyl)-1H-2-benzopyran-1-one] (**5i**)*: Yellow crystals, mp: 90°C; 61.7% yield; IR (KBr, υ, cm^{-1}): 1735 (γ lactone), 1600 (C=O), 3189 (OH), 1163 (OCH_3); Anal. Calcd $C_{41}H_{28}O_9$ (664.0 g): C, 74.09; H, 4.21; Found: C, 73.86; H, 4.65; ^{1}H NMR ($CDCl_3$)δ 3.99 (s, 6H, OCH_3), 7.35 (s, 1H, CH), 7.04–7.79 (m, 18H, aromatic protons), 8.39 (dd, 2H, CH) 12.70 (s, 1H, OH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 167.8 (C-OCH_3), 56 (O–CH_3), 38.7 (CH), 114.5 (CH), 130.7 (CH), 129 (C), 125.8 (C), 137(C), 128 (C), 130.2 (CH), 127.6 (CH), 134 (CH), 126.2 (CH), 154 (CH), 115 (CH), 130.5 (CH), 130.3 (C); ms: m/z: 662 (M^+-2, 3%), 647(1), 585(19), 508(11), 497(14), 384(24), 392(30), 145(36) and 135(49).

2.3j *4, 4′-(4-Methoxybenzylidene)-bis-[3-(4′-methoxy benzoyl)-1H-2-benzopyran-1-one] (**5j**)*: Yellow crystals, mp: 134°C; 60.0% yield; 1735 (γ lactone), 1626 (C=O), 1258 (OCH_3); Anal. Calcd $C_{42}H_{30}O_9$ (678.0 g): C, 74.33; H, 4.42; Found: C, 74.47; H, 4.68; ^{1}H NMR ($CDCl_3$)δ 4.16 (s, 9H, OCH_3), 7.45 (s, 1H, CH), 7.31–7.85 (m, 18H, aromatic protons), 8.40 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 167.4 (C-OCH_3), 56.2 (O –CH_3), 39 (CH), 114.6 (CH), 130.7 (CH), 129 (C), 125.5 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 158 (C–OCH_3), 57 (O-CH_3), 114 (CH), 130.2 (CH), 130 (C); ms: m/z: 678 (M^+, 17%), 398(2), 280(27), 146(70), 135(41) and 118(52).

2.3k *4, 4′-(4-Nitrobenzylidene)-bis-[3-(2′, 4′-dihyroxy benzoyl)-1H-2-benzopyran-1-one] (**5k**)*: Yellow crystals, mp: 75°C; 34.4% yield; 1702 (γ lactone), 1682 (C=O), 3320 (OH), 1590 (NO_2); Anal. Calcd $C_{39}H_{23}O_{12}N$ (697.0 g): C, 67.14; H, 3.29; N, 2.00; Found: C, 66.29; H, 3.57; N, 1.84; ^{1}H NMR ($CDCl_3$)δ 7.42 (s, 1H, CH), 6.82–8.19 (m, 16H, aromatic protons), 8.46 (d, 2H, CH), 11.60 (s, 2H, OH), 11.80 (s, 2H, OH) ; ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 165 (C-OH), 160 (C-OH), 33 (CH), 108.8 (CH), 103.4 (CH), 132 (CH), 116.5 (C), 125.5 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 145.4 (C –NO_2), 123.5 (CH), 130.1 (CH), 143.9 (C); ms: m/z: 698 (M^++1, 6%), 680(23), 617(8), 558(20), 540(34), 453(27), 415(20), 264(48), 173(39), 146(43) and 137(44).

2.3l *4, 4′-(4-Hydroxybenzylidene)-bis-[3-(2′, 4′-dihydroxy benzoyl)-1H-2-benzopyran-1-one] (**5l**)*: White crystals, mp: 126°C; 38.7% yield; 1742 (γ lactone), 1706 (C=O), 3310 (OH); Anal. Calcd $C_{39}H_{25}O_{11}$ (669.0 g): C, 69.95; H, 3.73; Found: C, 70.21; H, 3.70; ^{1}H NMR ($CDCl_3$)δ 7.47 (s, 1H, CH), 6.81–7.79 (m, 17H, aromatic protons), 8.38 (d, 2H, CH), 9.84 (s, 1H, OH), 12.53 (s, 2H, OH), 12.70 (s, 2H, OH) ; ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 165 (C-OH), 160 (C-OH), 32 (CH), 109 (CH), 103 (CH), 132.5 (CH), 117 (C), 125.5 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 154 (C-OH), 115.5 (CH), 130.5 (CH), 130.4 (C); ms: *m/z*: 668 (M^+-1, 1%), 618 (2), 541(6), 436(16), 387(45), 331(22), 282(17), 173(40), 146(57) and 77(92).

2.3m *4, 4′-(4-Methoxybenzylidene)-bis-[3-(2′, 4′-dihydroxy benzoyl)-1H-2-benzopyran-1-one] (**5m**)*: Yellow crystals, mp: 144°C; 35.2% yield; 1705 (γ lactone), 1695 (C=O), 3568 (OH); Anal. Calcd $C_{40}H_{26}O_{11}$ (683.0 g): C, 70.27; H, 3.95; Found: C, 69.84; H, 3.78; ^{1}H NMR ($CDCl_3$)δ 3.83 (s, 3H, OCH_3), 5.79 (s, 4H, OH), 7.42 (s, 1H, CH), 6.60–7.57 (m, 16H, aromatic protons), 8.42 (d, 2H, CH); ^{13}C NMR ($CDCl_3$)δ 163 (C=O, lactone), 187 (C=O, ketone), 134.3 (=C-O), 164.5 (C-OH), 159.9 (C-OH), 33.3 (CH), 108.8 (CH), 103.4 (CH), 132 (CH), 116.5 (C), 125.5 (C), 136.5 (C), 128.3 (C), 130 (CH), 127.6 (CH), 133.6 (CH), 126 (CH), 159 (C–OCH_3), 56 (O–CH_3), 114 (CH), 130.2 (CH), 130 (C); ms: *m/z*: 683 (M^+, 11%), 666 (8), 618(32), 541(10), 495(18), 439(5), 401(6) and 146(33).

3. Results and discussion

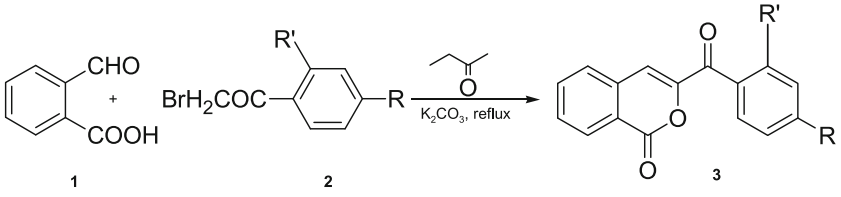
3.1 *Chemistry*

2-Carboxy benzaldehyde was refluxed with different substituted bromo acetophenones in presence of anhy. K_2CO_3 for 10–12 h, taking ethyl methyl ketone as solvent to get 3-aroyl isocoumarins (figure 1). Characterization of synthesized compounds was done by IR and NMR spectral studies. All the compounds in **3a–f** show absorption at 1735–1700 cm^{-1} for lactonic carbonyl and 1629–1543 cm^{-1} for ketonic carbonyl. Presence of C-Br in **3a** and –OH group in **3b** and **3c** is confirmed by signals at 680 cm^{-1} and 3528–3556 cm^{-1} (br.), respectively. NMR spectrum of **3a–3d** shows signals at δ 7.04–8.00 (m, 9H) for vinylic as well as aromatic protons, at δ 3.95 (s, OCH_3, 3H), with the proton at 8th position showing a characteristic doublet at around δ 8.34–8.44. Mass spectrum of **3a** (MW 328.9 g) shows M^+ peak at m/z 328.97, base peak at m/z 146 for $C_9O_2H_6$(isocoumarin moiety without any substitution) and m/z 174 for M^+–C_6H_4Br. Mass spectrum of **3d** (MW 266) shows M^+ peak at m/z 266.04, base peak at m/z 121 for M^+-$COC_6H_4OH$and m/z 146 for $C_9O_2H_6$(isocoumarin moiety without any substitution).

3-Aroyl isocoumarins **3** formed were then condensed with different aryl aldehydes **4** in the ratio 2:1 in ethanol to get bis-(1*H*-2-benzopyran-1-one) derivatives **5a–m** (figure 2). **5a** shows IR signals for –OH, lactonic carbonyl and ketonic carbonyl, respectively. NMR spectrum of **5a** shows signals at δ 5.75–7.50 (s, 1H, CH), 6.90–8.10 (m, 18H, aromatic protons), 8.25–8.45 (d, 2H, CH) 10.00–10.50 (s, 2H, OH). Mass spectrum of **5a** gives m/z peak at 480, 410, 390, 375, 298, 266, 145, 121, 118 and 77.

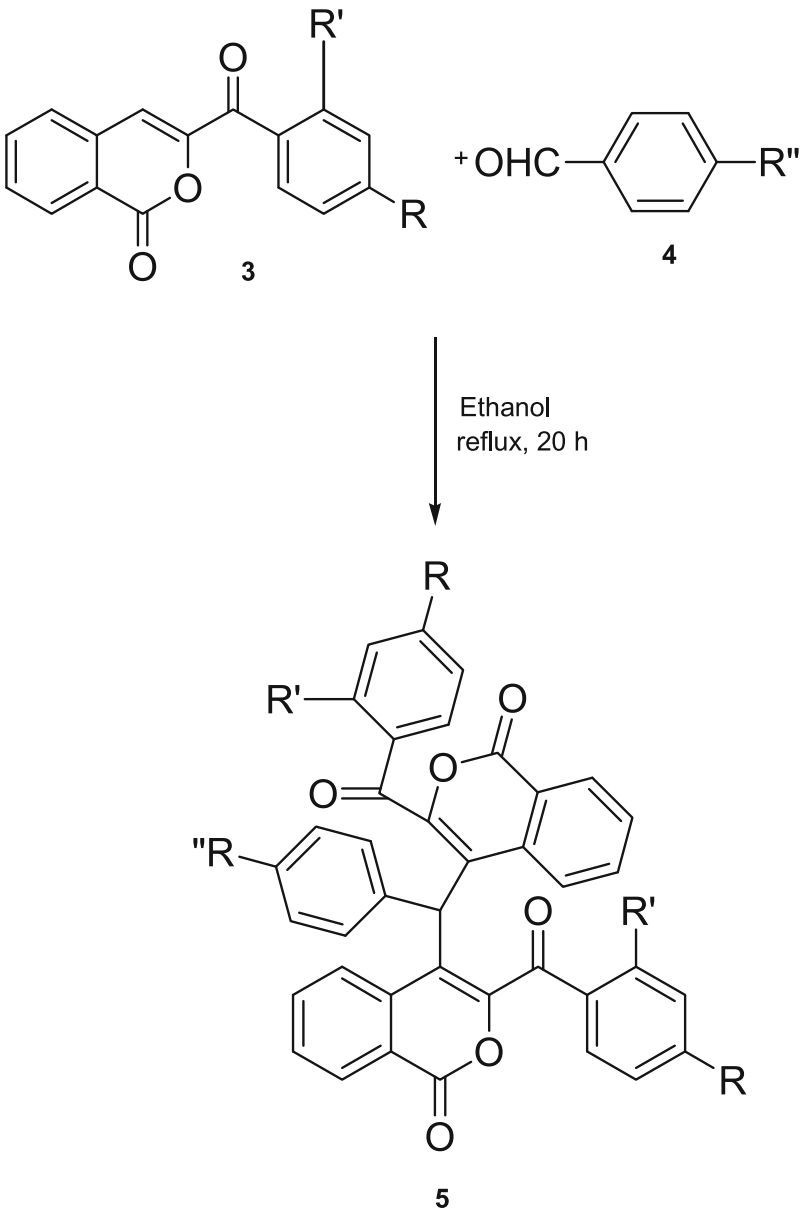
3.2 *Pharmacology*

Some of the prepared compounds **3a–b**, **d**, **5b–d** were tested *in vitro* against bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using serial agar dilution (cup plate method)[20] and fungal strains of *Fusarium pallidoroseum* and *Chaetonium* using Potato Dextrose Agar medium (Poisoned Food Technique),[21] respectively.



R = Br , R' = H (**3a**)
R = OH, R' = H (**3b**)
R = OH, R' = OH (**3c**)
R = OCH_3, R' = H (**3d**)
R = Ph, R' = H (**3e**)
R = Benzofuran, R' = H (**3f**)

Figure 1. Synthetic pathway for the preparation of compounds **3a–f**.



R = OH, R' = H, R" = Cl (**5a**)
R = OH, R' = H, R" = H (**5b**)
R = OH, R' = H, R" = NO_2 (**5c**)
R = OH, R' = H, R" = OCH_3 (**5d**)
R = Br, R' = H, R" = NO_2 (**5e**)
R = Br, R' = H, R" = OH (**5f**)
R = Br, R' = H, R" = H (**5g**)
R = OCH_3, R' = H, R" = NO_2 (**5h**)
R = OCH_3, R' = H, R" = OH (**5i**)
R = OCH_3, R' = H, R" = OCH_3 (**5j**)
R = OH, R' = OH, R" = NO_2 (**5k**)
R = OH, R' = OH, R" = OH (**5l**)
R = OH, R' = OH, R" = OCH_3 (**5m**)

Figure 2. Synthetic pathway for the preparation of compounds **5a–m**.

Table 1. Antimicrobial activity.

Compound	Zone of inhibition (mm)		% Growth of inhibition	
	S. aureus	*E .coli*	*F. pallidoroseum*	*Chaetonium*
3a	16	14	13.75	6.83
3b	15	11	29.60	17.76
3d	11	14	48.97	43.10
5b	15	14	64.87	70.60
5c	17	14	57.34	66.64
5d	15	15	64.08	70.00
Control (DMF)	0	10	-	-
Ampicillin	15	5	-	-

Table 2. Analgesic activity.

Compound	Dose (mg/kg) body weight	Average (± SE) reaction time (s) time after drug treatment (min)			
		0	30	60	90
Control	50	3.01	3.20	3.10	3.02
Standard	50	3.09	5.25	7.75	9.00
3a	50	1.32	3.28	3.69	4.77
3b	50	2.46	3.00	3.04	3.88
3d	50	3.62	3.97	4.01	5.35
5b	50	4.00	4.53	5.98	7.44
5c	50	3.53	4.20	5.68	6.52
5d	50	3.41	4.73	5.05	6.93

The zone of inhibition of all compounds was found to show moderate activity against Gram-negative i.e., *Escherichia coli* bacteria and significant activity was seen with all compounds against Gram-positive bacteria *S. aureus*. Among all, excellent result was with **5c** where $-NO_2$ group is substituted to isocoumarin moiety apart from two –OH groups attached to aroyl group. Promising effect was with **3a**, where **–Br** was substituted to aroyl group. Presence of hydroxyl group in aroyl moiety and $-NO_2$ in isocoumarin moiety has enhanced antibacterial activity.

The pattern of the result for antifungal activity of the tested compounds was quite different from antibacterial activity. Compound **3a–b**,**d** were found to be weakly or moderately active against both fungi where only one isocoumarin moiety was present, but the compounds **5b–d** where two isocoumarin moieties exist, showed almost double the activity among all compounds. The conclusion might be drawn that isocoumarin moiety plays an important role in showing antifungal activity rather than the substituent group present in it as seen in antibacterial activity.

The results of antimicrobial screening of selected new compounds are summarized in table 1.

Analgesic activity of the compounds was determined by tail flick method[22] on mice of either sex. Gum acacia (2%) was used as control, Analgin was the standard drug. 3-Aroyl isocoumarins fare badly in reaction time when compared to the standard drug. The response time is tremendous, almost equivalent to standard drug when two six-membered lactone rings are present together, irrespective of the other groups present in them. This shows that the lactone ring in itself is useful in for biological applications.

The results are shown in table 2.

4. Conclusion

In conclusion, new bis-(1*H*-2-benzopyran-1-one) derivatives were prepared from easily accessible starting materials in a single step. The preliminary *in vitro* test results of these compounds against the four studied micro-organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Fusarium pallidoroseum* and *Chaetonium* shows significant activity. All the tested compounds showed good analgesic action also.

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