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Biscoumarin Analogs: Synthesis, α-Glucosidase Inhibitory Potential and Molecular Docking Study

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Eighteen biscoumarin analogs **(1-18)** were synthesized in moderately good yields and characterized by various spectroscopic methods. The biscoumarin analogs were further evaluated for α-glucosidase inhibitory potential. Amongst this series of 18 analogs, 15 analogs showed outstanding α-glucosidase inhibititory activity with IC_{50} values ranging from 14.39 ± 0.52 to 230.22 ± 0.83 μM when compared with the standard acarbose of IC_{50} value of 774.5 ± 1.94 μM, in which analog **4** was observed as the most active analog. Molecular docking study was carried out to understand the binding interaction of the compounds with the active site of α-glucosidase. Analog **4** was found to possess the most interaction with various proteins. This is in line with the findings in the α-glucosidase assay.

Key words: Synthesis; biscoumarin; α-glucosidase inhibition; Molecular docking study; SAR

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α-Glucosidase is involved in carbohydrate metabolism and has a crucial function in diabetes, viral infection, and cancer. Anti-diabetic agents that are used in clinical practice, such as acarbose [1], voglibose, and miglitol [2] competitively inhibit *α*-glucosidase in the brush border of the small intestine, which consequently delay the hydrolysis of carbohydrates and alleviate postprandial hyperglycemia. However, the continuous administration of these agents may cause several adverse effects, such as diarrhea, abdominal discomfort, flatulence [3, 4, 5] and hepatotoxicity [6]. Therefore, developing novel α-glucosidase inhibitors lacking these liabilities are necessary, given the therapeutic challenge of type II diabetes mellitus.

Biscoumarins have received considerable attention in the past few years for their versatile biological and medicinal properties, such as anticoagulant [7, 8], antidiabetic [9], urease inhibitor [10], anticancer [11], and antibacterial [12, 13] activities. A number of biscoumarins significantly inhibit c-Met phosphorylation in BaF3/TPR-Met and EBC-1 NSCLC cell lines[14]. Recently, biscoumarin derivatives have also been found to significantly inhibit bladder urothelial cancer cells growth through anti-proliferation and induce apoptosis [15]. Currently, our group has reported a number of α-glucosidase inhibitors such as benzothiazole, oxadiazole bearing benzohydrazide, and biscoumarin thiourea derivatives [16, 17, 18]. In this report, we report α-glucosidase inhibitory activity of new biscoumarin analogs and their molecular docking analysis.

MATERIALS AND METHODS

1. General

Melting point was taken on Buchi M-560 melting point instrument and was uncorrected. IR spectra were recorded on Spectrum One FT-IR spectrometer (Perkin Elmer), using KBr discs and values were signified in cm^{-1}. ^{1}H and ^{13}C-NMR spectra were measured on Bruker 500 Ultrashield Plus NMR (500 MHz) in DMSO-*d*6 as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts were expressed as ppm. ESI MS were determined on Agilent 6330 Ion Trap using positive/negative mode at Faculty of Pharmacy, UiTM Puncak Alam, Malaysia.

2. General Method for the Synthesis of Compounds 1-18

Biscoumarin analogs **1-18** were synthesized by stirring the mixture of 6,7-dimethyl-4-hydroxycoumarin (1

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mmol) and substituted aromatic aldehydes (0.5 mmol) in water and 10 mmol triethyl ammonium bromide (TEAB)(Khan *et al*., 2014). The reaction mixture was refluxed for 24 hours. Completion of the reaction was monitored by periodic TLC. After completion of the reaction, the mixture was filtered and then washed with distilled water affording pure products in high yields. The structures of compounds **1-18** were characterized by using different spectroscopic techniques, including ^{1}H and ^{13}C NMR, FT-IR and ESI mass spectroscopy and melting point.

2.1. 3,3'-((2-chlorophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chrome-2-one) (1)

Off-white solid; Yield 78%; m.p. 239.7°C. IR (KBr) ν_{max}, cm^{-1}; 3063, 2923, 1620, 1559, 1442, 1341, 1289, 1254, 1226, 1184, 1162, 1118, 1071, 1040, 862, 830, 798, 749. ^{1}H-NMR (500 MHz, DMSO) δ 7.66 (s, 2H), 7.32 (s, 2H), 7.22 (t, *J* = 6.5 Hz, 2H), 7.16 (s, 2H), 6.21 (s, 1H), 2.30 (s, 6H), 2.25 (s, 6H).^{13}C-NMR (126 MHz, DMSO) δ 164.7, 164.6, 151.0, 142.0, 139.0, 133.3, 132.6, 130.3, 129.9, 128.1, 127.0, 124.0, 116.6, 115.3, 103.9, 36.5, 20.0, 19.3. ESI-MS: 501.1110 (M^{-}).

2.2. 3,3'-((4-chlorophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (2)

Off-white solid; Yield 77%; m.p. 242.8°C; IR (KBr) ν_{max}, cm^{-1}; 3051, 2921, 1673, 1631, 1566, 1491, 1449, 1359, 1308, 1255, 1184, 1131, 1096, 1070, 1017, 835, 789. ^{1}H-NMR (500 MHz, DMSO) δ 7.64 (s, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.17 (s, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.30 (s, 1H), 2.31 (s, 6H), 2.26 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 166.0, 165.6, 151.1, 142.1, 132.7, 129.1, 128.4, 124.3, 116.8, 115.7, 103.6, 35.9, 20.1, 19.3. ESI-MS: 501.1114 (M^{-}).

2.3. 3,3'-((2,4-dichlorophenyl) methylene) bis (4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (3)

Off-white solid; Yield 81%; m.p. 230.2°C; IR (KBr) ν_{max}, cm^{-1}; 2922, 1663, 1623, 1560, 1452, 1298, 1182, 1124, 1067, 1013, 865, 835, 801, 786. ^{1}H-NMR (500 MHz, DMSO) δ 7.64 (s, 2H), 7.43 (d, *J* = 1.3 Hz, 1H), 7.35 – 7.24 (m, 2H), 7.13 (s, 2H), 6.10 (s, 1H), 2.30 (s, 6H), 2.25 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 165.5, 165.3, 164.3, 151.1, 141.7, 139.1, 134.1, 132.4, 131.9, 131.4, 129.1, 126.9, 124.1, 116.8, 115.7, 103.4, 36.4, 20.0, 19.3. ESI-MS: 535.0723 (M^{-}).

2.4. 3,3'-((2-fluorophenyl)methylene)bis(4-hydroxy -6,7-dimethyl-2H-chromen-2-one) (4)

Off-white solid; Yield 76%; m.p. 292.7°C; IR (KBr) ν_{max}, cm^{-1}; 2954, 1652, 1565, 1489, 1452, 1365, 1314, 1230, 1184, 1129, 1069, 1015, 890, 872, 792, 757. ^{1}H-NMR (500 MHz, DMSO) δ 7.65 (s, 2H), 7.23 (bs, 2H), 7.16 (s, 2H), 7.06 (dd, *J* = 18.5, 9.2 Hz, 2H), 6.35 (s, 1H), 2.30, 2.26 (2s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 165.1, 164.9, 151.0, 141.9, 132.5, 130.0, 128.2, 129.2, 124.2, 124.1, 116.8, 115.7, 115.5, 115.4, 103.4, 32.4, 20.1, 19.4. ESI-MS: 485.1408 (M^{-}).

2.5. 3,3'-((4-fluorophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (5)

Off-white solid; Yield 78%; m.p. 297.0°C; IR (KBr) ν_{max}, cm^{-1}; 2975, 1664,1628, 1567, 1506, 1448, 1357, 1309, 1221, 1184, 1162, 1130, 1106, 1068, 1016, 870, 823, 792. ^{1}H-NMR (500 MHz, DMSO) δ 7.63 (s, 2H), 7.16 (s, 2H), 7.07 (d, *J* = 41.8 Hz, 2H), 7.00 (d, *J* = 8.1 Hz, 2H), 6.27 (s, 1H), 2.31 (s, 6H), 2.27 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 166.3, 165.5, 151.1, 141.8, 137.0, 132.4, 128.9, 128.86, 124.34, 116.7, 116.2, 115.1, 114.9, 103.8, 35.8, 20.1, 19.3. ESI-MS: 485.1407 (M^{-}).

2.6. 3,3'-(o-tolylmethylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (6)

Off-white solid; Yield 72%; m.p. 258.9°C; IR (KBr) ν_{max}, cm^{-1}; 2953, 1655, 1627, 1563, 1451, 1361, 1312, 1255, 1230, 1183, 1128, 1111, 1067, 1014, 860, 833, 804, 785, 746. ^{1}H-NMR (500 MHz, DMSO) δ 7.63 (s, 2H), 7.20 (bs, 1H), 7.15 (s, 2H), 7.06 (s, 3H), 6.11 (s, 1H), 2.31 (s, 6H), 2.26 (s, 6H), 2.05 (s, 3H). ^{13}C-NMR (126 MHz, DMSO) δ 165.40, 164.9, 151.0, 141.7, 136.4, 132.5, 130.8, 128.1, 126.3, 125.7, 124.2, 116.8, 115.7, 103.9, 35.9, 20.1, 19.9, 19.3. ESI-MS: 481.1659 (M^{-}).

2.7. 3,3'-(m-tolylmethylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (7)

Off-white solid; Yield 81%; m.p. 273.1°C; IR (KBr) ν_{max}, cm^{-1}; 2920, 1655, 1627, 1567, 1449, 1355, 1310, 1184, 1129, 1107, 1070, 1015, 874, 832, 813, 788. ^{1}H-NMR (500 MHz, DMSO) δ 7.65 (s, 2H), 7.18 (s, 2H), 7.10 (t, *J* = 7.2 Hz, 1H), 6.96 (d, *J* = 7.1 Hz, 1H), 6.90 (d, *J* = 7.9 Hz, 2H), 6.29 (s, 1H), 2.32 (s, 6H), 2.27 (s, 6H), 2.20 (s, 3H). ^{13}C-NMR (126 MHz, DMSO) δ 165.9, 165.7, 151.1, 142.0, 140.6, 137.4, 132.6, 128.4, 127.6, 126.7, 124.3, 116.8, 115.89, 103.9, 36.2, 21.7, 20.1, 19.3. ESI-MS: 481.1658 (M^{-}).

2.8. 3,3'-(p-tolylmethylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (8)

Off-white solid; Yield 79%; m.p. 275.9°C; IR (KBr) ν_{max}, cm^{-1}; 2919, 1674, 1630, 1567, 1512, 1443, 1355, 1306, 1254, 1131, 1069, 887, 775. ^{1}H-NMR (500 MHz, DMSO) δ 7.62 (s, 2H), 7.15 (s, 2H), 6.99 (dd, *J* = 18.9, 7.4 Hz, 4H), 6.25 (s, 1H), 2.31 (s, 6H), 2.27 (s, 6H), 2.24 (s, 3H). ^{13}C-NMR (126 MHz, DMSO) δ 165.6, 151.1, 141.7, 137.8, 132.4, 129.0, 127.0, 124.3, 118.3, 116.7,

103.9, 36.0, 20.9, 20.1, 19.3. ESI-MS: 481.1663 (M^-).

2.9. 3,3'-((3-hydroxyphenyl)methylene)bis(4-hydroxy -6,7-dimethyl-2H-chromen-2-one) (9)

Off-white solid; Yield ; m.p. 278.9°C; IR (KBr) v_{max}, cm^{-1}; 3336, 2975, 1655, 1624, 1567, 1500, 1448, 1362, 1308, 1272, 1186, 1161, 1133, 1070, 1017, 879, 818, 794. ^{1}H-NMR (500 MHz, DMSO) δ 7.67 (s, 2H), 7.20 (s, 2H), 7.01 (s, 1H), 6.55 (s, 3H), 6.27 (s, 1H), 2.32 (s, 6H), 2.28 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 165.7, 165.6, 157.7, 151.0, 142.2, 141.7, 132.8, 129.4, 124.3, 117.8, 116.8, 115.6, 114.05, 113.1, 103.9, 36.1, 20.1, 19.3. ESI-MS: 483.1452 (M^-).

2.10. 3,3'-((4-hydroxyphenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (10)

Off-white solid; Yield 72%; m.p. 232.1 °C; IR (KBr) v_{max}, cm^{-1}; 3460, 2946, 1655, 1628, 1563,1515, 1436, 1358, 1256, 1181, 1133, 1070, 1016, 871, 838, 793. ^{1}H-NMR (500 MHz, DMSO) δ 7.65 (s, 2H), 7.19 (s, 2H), 6.89 (d, *J* = 7.8 Hz, 2H), 6.63 (d, *J* = 7.8 Hz, 2H), 6.21 (s, 1H), 2.32 (s, 6H), 2.28 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 165.7, 165.3, 155.8, 151.0, 142.1, 132.8, 128.0, 124.2, 116.8, 115.5, 115.4, 104.3, 35.5, 20.1, 19.3. ESI-MS: 483.1456 (M^-).

2.11. 3,3'-((2-hydroxy-4-methoxyphenyl)methylene) bis (4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (11)

Off-white solid; Yield 83%; m.p. 269.8°C; IR (KBr) v_{max}, cm^{-1}; 3229, 2946, 1682, 1611, 1521, 1308, 1197, 1125, 1029, 845, 707. ^{1}H-NMR (500 MHz, DMSO) δ 7.52 (s, 2H), 6.99 (s, 2H), 6.18 – 6.17 (m, 2H), 6.05 (s, 1H), 3.62 (s, 3H), 2.27(s, 6H), 2.24 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 167.8, 165.0, 158.2, 156.1, 151.2, 139.8, 131.1, 129.9, 124.6, 122.2, 118.4, 116.2, 103.8, 103.1, 101.4, 55.0, 32.6, 20.0, 19.3. ESI-MS: 513.1554 (M^-).

2.12. 3,3'-((4-methoxyphenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one)(12)

Off-white solid; Yield 79%; m.p. 255.3°C; IR (KBr) v_{max}, cm^{-1}; 2936, 1674, 1630, 1567, 1509, 1450, 1360, 1303, 1248, 1177, 1131, 1067, 1035, 886, 768. ^{1}H-NMR (500 MHz, DMSO) δ 7.64 (s, 2H), 7.18 (s, 2H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.25 (s, 1H), 3.74 (s, 3H), 2.31 (s, 6H), 2.27 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 165.7, 165.6, 157.8, 151.0, 142.1, 132.7, 131.9, 128.1, 124.2, 116.8, 115.7, 114.0, 104.1, 55.5, 35.5, 21.7, 19.3. ESI-MS: 497.1609 (M^-).

2.13. 3,3'-((3,4-dimethoxyphenyl)methylene)bis(4-hyd roxy-6,7-dimethyl-2H-chromen-2-one) (13)

Off-white solid; Yield 75%; m.p. 248.2°C; IR (KBr) v_{max}, cm^{-1}; 2933, 1660, 1627, 1561, 1509, 1450, 1358, 1331, 1306, 1254, 1185, 1143, 1029, 892, 862, 788. ^{1}H-NMR (500 MHz, DMSO) δ 7.63 (s, 2H), 7.15 (s, 2H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.66 (s, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.23 (s, 1H), 3.70 (s, 3H), 2.31 (s, 6H), 2.27 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 166.2, 165.6, 151.1, 149.0, 147.5, 141.8, 133.3, 132.5, 124.3, 119.3, 116.7, 116.1, 112.2, 111.8, 104.0, 56.0, 36.0, 20.1, 19.3. ESI-MS: 527.1781 (M^-).

2.14. 3,3'-((2-nitrophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (14)

Off-white solid; Yield 81%; m.p. 247.5°C; IR (KBr) v_{max}, cm^{-1}; 2922, 1620, 1559, 1527, 1447, 1354, 1289, 1228, 1184, 1126, 1071, 1013, 962, 886, 866, 829, 797, 783. ^{1}H-NMR (500 MHz, DMSO) δ 7.65 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.13 (s, 2H), 6.54 (s, 1H), 2.30 (s, 6H), 2.25 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 166.0, 164.3, 151.2, 150.0, 141.7, 135.2, 132.3, 132.3, 130.1, 127.5, 124.4, 124.2, 116.7, 115.8, 103.0, 34.4, 20.1, 19.3. ESI-MS: 512.1355 (M^-).

2.15. 3,3'-((3-nitrophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (15)

Off-white solid; Yield 74%; m.p. 275.8°C; IR (KBr) v_{max}, cm^{-1}; 3059, 2935, 1615, 1537, 1459, 1335, 1238, 1100, 959, 884, 785. ^{1}H-NMR (500 MHz, DMSO) δ 8.02 (d, *J* = 7.6 Hz, 1H), 7.89 (s, 1H), 7.61 (s, 2H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.15 (s, 2H), 6.37 (s, 1H), 2.31 (s, 6H), 2.26 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 167.2, 165.2, 151.3, 148.3, 144.8, 141.7, 134.4, 132.3, 129.9, 124.5, 121.6, 121.0, 116.7, 116.6, 102.9, 36.7, 20.1, 19.4. ESI-MS: 512.1353 (M^-).

2.16. 3,3'-((4-nitrophenyl)methylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (16)

Off-white solid; Yield 81%; m.p. 268.4°C; IR (KBr) v_{max}, cm^{-1}; 3051, 2922, 1659, 1624, 1567, 1520, 1450, 1350, 1183, 1133, 1111, 1070, 1012, 876, 855, 793. ^{1}H-NMR (500 MHz, DMSO) δ 8.09 (d, *J* = 8.3 Hz, 2H), 7.63 (s, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.16 (s, 2H), 6.40 (s, 1H), 2.31 (s, 6H), 2.26 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 166.5, 165.4, 151.2, 150.2, 146.1, 142.1, 132.6, 128.5, 124.4, 123.6, 116.8, 116.0, 103.2, 37.0, 20.1, 19.3. ESI-MS: 512.1356 (M^-).

2.17. 3,3'-(pyridin-3-ylmethylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (17)

Off-white solid; Yield 84%; m.p. 284.2°C; IR (KBr) v_{max}, cm^{-1}; 3446, 2919, 1680, 1624, 1559, 1466, 1389,

1321, 1199, 1106,1000, 850, 833, 787. ^{1}H-NMR (500 MHz, DMSO) δ 8.70 (d, *J* = 4.6 Hz, 1H), 8.57 (s, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 7.93 (m, 1H), 7.53 (s, 2H), 7.10 (s, 2H), 6.39 (s, 1H), 2.29(s, 6H), 2.24 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 168.7, 164.8, 151.5, 145.1, 143.7, 141.2, 140.7, 139.4, 131.8, 127.0, 124.7, 117.5, 116.6, 101.5, 35.1, 20.1, 19.3. ESI-MS: 468.1453 (M^-).

2.18. 3,3'-(pyridin-4-ylmethylene)bis(4-hydroxy-6,7-dimethyl-2H-chromen-2-one) (18)

Off-white solid; Yield 80%; m.p. 308.2°C; IR (KBr) ν_{max}, cm^{-1}; 3440, 2880, 1682, 1624, 1561, 1456, 1390, 1188, 1108, 1003, 860, 831, 784. ^{1}H-NMR (500 MHz, DMSO) δ 8.64 (d, *J* = 5.3 Hz, 2H), 7.72 (d, *J* = 5.1 Hz, 2H), 7.54 (s, 2H), 7.11 (s, 2H), 6.42 (s, 1H), 2.30 (s, 6H), 2.25 (s, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 168.8, 165.7, 164.8, 151.5, 141.5, 141.3, 131.8, 125.5, 124.7, 117.5, 116.6, 101.9, 38.3, 20.1, 19.2. ESI-MS: 468.1456 (M^-).

3. Baker's Yeast α-Glucosidase Inhibition Assay

The enzyme inhibition was evaluated according to the method previously reported by Taha *et al.* (2015a, 2015b) with slight modifications. Various concentrations of the test compounds (10 µL) were dissolved in DMSO (in the range of 200 – 6.25 µg/mL) and premixed with 95 µL of 50 mM phosphate buffer (pH 6.8). Then, 25 µL of the enzyme (0.0625 U/mL) in phosphate buffer saline was added into each well and the plate was incubated at 37°C for 10 min. Afterward, 25 µl of PNPG in phosphate buffer saline (5 mM) was added and the reading of the plate was taken by using a microplate reader (Spectrostar Nano BMG Labtech, Germany). The reaction mixture was then incubated at 37°C for 30 min and change in absorbance at 405 nm was monitored up to 30 min. For negative control, the test samples were replaced with 10 µL of DMSO and acarbose was used as positive control. All experiments were triplicated and the results were expressed as mean ± S.E.M of three determinations. The percentage (%) inhibition of α-glucosidase inhibitory activity was calculated using the equation: where $\Delta A_{control}$ and ΔA_{sample} are the different absorbance of control and sample, respectively, at time t_{30} and t_0.

$$\% \text{ Inhibition} = \frac{\Delta A\ control\ -\ \Delta A sample}{\Delta A\ control} \times 100$$

4. Molecular Docking Experiment

Protein-ligand docking study was carried out to dock biscoumarin analogs against α-glucosidase with the following communications; Intel(R) xenon(R) CPU E5620@2.40GHz system having 3.8GB RAM with the open 11.4 (X 86_64) operating platform using the Molecular Operating Environment (MOE 2010.11) software package. The crystallographic structure of α-glucosidase of *Saccharomyces cerevisiae* is not available and only few homology models have been reported [19, 20, 21, 22]. So, we developed the 3D model for α-glucosidase by comparative homology modeling technique using the same etiquette as described by [23]. The primary sequence was retrieved from UniProt (Access code P53341). The crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB code 3AJ7; Resolution 1.30 Å) with 72.4% of sequence identity with the target was selected as a template [24] (Barakat *et al*., 2015). The 3D structure of α-glucosidase of *Saccharomyces cerevisiae* was predicted using MOE homology modelling tools. The predicted model was then subjected to energy minimization up to 0.05 gradient.

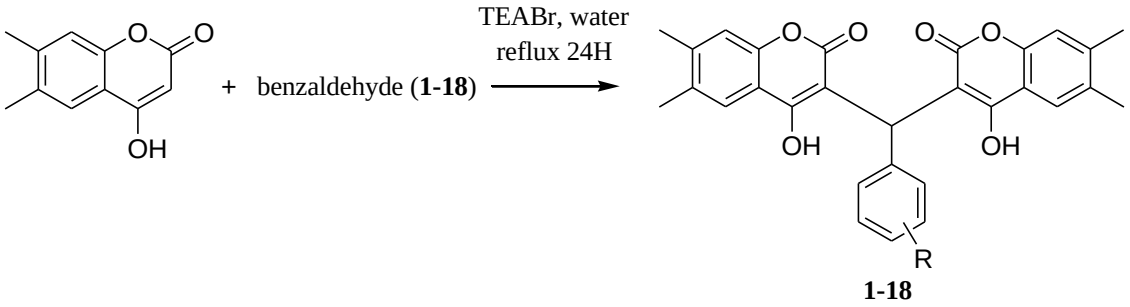
Before docking, ligands and protein were prepared using MOE v2010.11. The 3D structure of each compound was built by using Molecular Builder Module program implemented in MOE and saved as a (.mdb) file for molecular docking. Subsequently, the energy of the compounds was minimized up to 0.05 Gradient using MMFF 94x force field. Energy minimization of the compounds was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was done prior to docking using Protonate 3D tools. Protonation was followed by energy minimization up to 0.05 Gradient using Amber 99 force field. All the compounds were then docked into the developed modelled protein of α-glucosidase. The top-ranked docked conformation of each compound was analyzed of its binding mode in the active site of α-glucosidase using Pymol.

RESULTS AND DISCUSSION

1. Synthesis of Biscoumarin Derivatives

In this work, a new series of biscoumarins were synthesized in moderately good yields, according to previous reports[9]. We began the synthesis of biscoumarin analogs **1-18** (Scheme 1) by stirring a mixture of 6,7-dimethyl-4-hydroxycoumarin (2 mmol) and substituted aromatic aldehydes (1 mmol) in water and a catalytic amount of tetraethylammonium bromide (TEAB). The formation of biscoumarins **1-18** was prompted with aldol condensation of 6,7-dimethyl-4-hydroxycoumarin (2 mmol) and substituted aromatic aldehyde linkers (1 mmol), followed by dehydration of the aldol product to give a chromone. Subsequent *in situ* reaction of the chromone with another 6,7-dimethyl-4-hydroxycoumarin present in excess gave dimeric coumarin derivatives **1–18** bearing an aryl substituent in the central methylene linker. The structures of compounds **1-18** were characterized by using different

spectroscopic techniques such as ^{1}H and ^{13}C NMR, FT-IR and ESI mass spectroscopy and their melting points.



Scheme 1. Synthesis of biscoumarin derivatives (1-18)

2. α-Glucosidase Inhibitory Activity

Amongst the series of 18 biscoumarin analogs, 15 compounds showed variable degrees of α-glucosidase inhibition with IC_{50} values ranging between 14.39±0.52 and 230.22±0.83 μM when compared with the standard acarbose with IC_{50} value of 774.5±1.94 μM. Compounds **1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 16**, and **17** showed outstanding inhibiting properties with IC_{50} values of 89.47±0.31, 15.91±0.56, 40.94±1.34, 14.39±0.52, 230.22±0.83, 20.72±0.76, 178.23±0.65, 53.66±1.19, 18.58±0.68, 126.33±0.43, 34.06±1.13, 31.16±1.07, 22.61±0.57, 19.17 ± 0.72, and 115.02±0.43 μM, respectively, which are many folds better than the standard acarbose.

The structure-activity relationship was mainly based upon by bringing about differences of substituents in the aldehydic phenyl part (Table 1). The most active analog amongst the series was compound **4**, having 2-fluoro at the aldehydic phenyl part (IC_{50} = 14.39±0.52 μM), while the second most active analog was compound **2**, having 4-chloro at the aldehydic phenyl part (IC_{50} = 15.91 ± 0.56 μM). Both of the substituents are electron-withdrawing groups which might play some role in affecting the inhibitions. If we compare compound **4** with compound **5,** a 4-fluoro analog (IC_{50} = 230.22 ± 0.83 μM), compound **4** would be found to be superior, which shows that the position of the substituents also plays a role in this inhibition.

If we compare analogs **1**(IC_{50} = 89.47 ± 0.31 μM), **2**(IC_{50} = 15.91 ± 0.56 μM), and **3** (IC_{50} = 40.94 ±1.34 μM), all three analogs possess the presence of chloro groups, but the arrangement of the chloro groups are different, which confirms that differences in position of substituents greatly affect the inhibitory potential of the compounds. In this study, we observed that both Electron Withdrawing Group (EWG) and Electron Donating Group (EDG) in the aldehydic phenyl part showed inhibiting potential but there exists a slight difference in potential as illustrated in **Table 1**, which was mainly affected by the positioning of substituents, as well as in some cases the number of substituents. In order to understand the binding interactions of the most active analogs, molecular docking has been utilized.

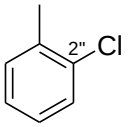
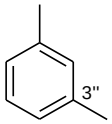
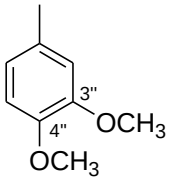
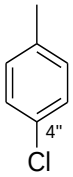
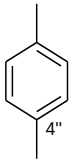
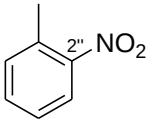
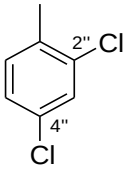
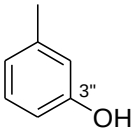
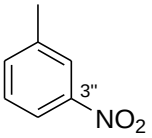
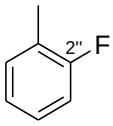
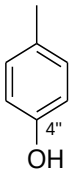
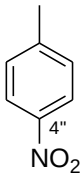
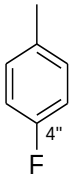
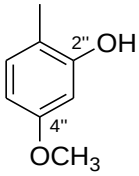
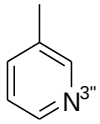
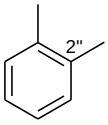
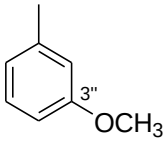
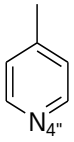
3. Molecular Operating Environment

MOE-Dock was used to explore the binding modes of biscoumarin analogs within the active site of α-glucosidase. To predict the correct conformations and to obtain energy minimizing structures, compounds were allowed to be flexible. From the docking simulation, it was observed that all the compounds fitted well in the binding cavity of α-glucosidase.

In our series of compounds, the most active was compound **4**, having fluorine (F) attached at *ortho* position of the aromatic ring, which showed good interaction with the active site residues, i.e., Lys155 and Arg312 (Figure 1). Compound **4** also formed arene-cation bonding with residues Lys155 and Arg312. Additionally, Arg 312 also showed hydrogen bonding with carbonyl oxygen of pyran moiety of the compound.

Compound **5** with fluorine attached at *para* position exhibited a single interaction with Arg312 (Figure 2a). A somewhat similar binding mode was observed for chloro substituted analogs, i.e., compounds **1, 2**, and **3**. As for methyl substituted compounds, compound **7** showed less activities and poor interactions with the active residues of α-glucosidase, while compound **8** was not active (Table 1). However, compound **6** with methyl attached at *ortho* position of the aromatic ring, two arene-cation interactions were established with Arg 312 and His 279 of the enzyme (Figure 2b).

Table 1. Inhibition of α-glucosidase by biscoumarin 6,7-dimethyl derivatives

No	Substituent	IC_{50}±SEM (μM)	No	Substituent	IC_{50}±SEM (μM)	No	Substituent	IC_{50}±SEM (μM)
1		89.47±0.31	7		178.23±0.65	13		34.06±1.13
2		15.91±0.56	8		NA	14		31.16±1.07
3		40.94±1.34	9		53.66±1.19	15		NA
4		14.39±0.52	10		18.58±0.68	16		22.61±0.57
5		230.22±0.83	11		126.33±0.43	17		19.17 ± 0.72
6		20.72±0.76	12		NA	18		115.02±0.43

*NA= not active

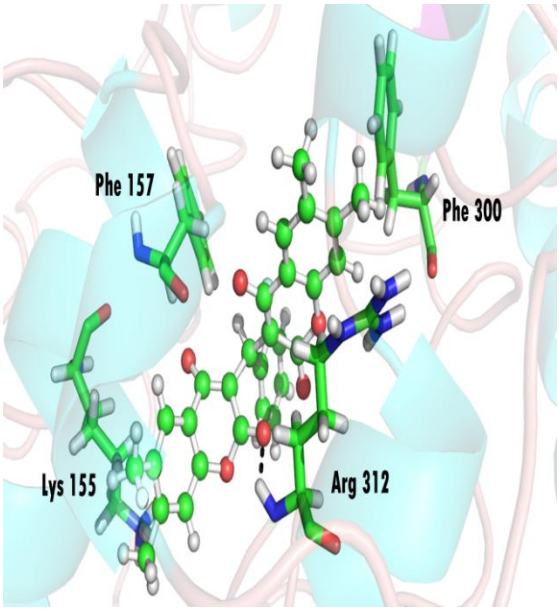


Figure 1. Docking pose of compound **4** in the active site

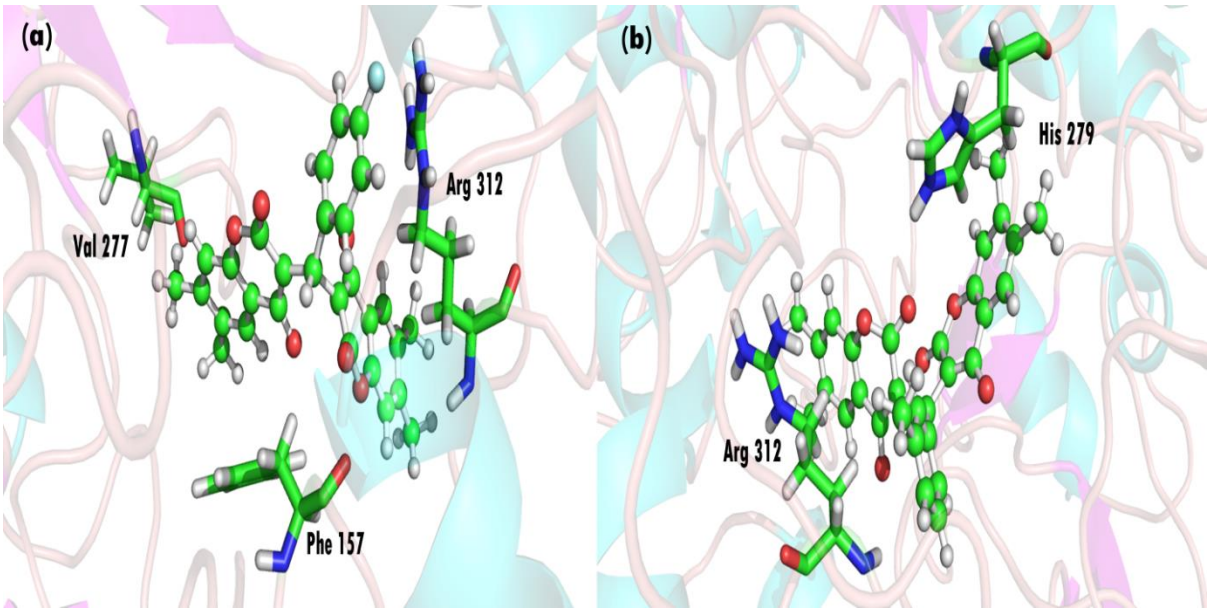


Figure 2. Docking pose of compounds **5** and **6** in the active site

CONCLUSION

As a conclusion, eighteen biscoumarin analogs (**1-18**) have been successfully synthesized and evaluated for their α-glucosidase inhibitory potential. Fifteen analogs showed variable degrees of α-glucosidase inhibition with IC_{50} values ranging from 14.39 ± 0.52 to 230.22 ± 0.83 μM while three analogs (**8, 15, 18**) were not active. Analogs **2, 4, 10**, and **17** were found to possess good inhibitory activity, in which, **4** showed the best activity. The molecular docking prediction showed that analog **4** has the most interaction with the proteins, which further supported the results in the α-glucosidase assay.

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