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ORIGINAL ARTICLE

Access to the substituted benzyl-1,2,3-triazolyl hesperetin derivatives expressing antioxidant and anticancer effects

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Abstract Azide–alkyne cycloaddition was attempted to generate a flavanone hesperetin based phenyl substituted 1,2,3-triazolyls as semi-synthetic natural product derivatives utilizing copper-catalyzed click chemistry. All final compounds were analyzed for their *in vitro* antioxidant abilities using DPPH and ABTS bioassay. Moreover, cancerous cell inhibitory prospect of titled compounds was screened against cervical cancer cell lines, HeLa and CaSki and an ovarian cancer cell line SK-OV-3 implementing SRB assay. Bearable toxicity of **6a-s** was examined employing Madin–Darby canine kidney (MDCK) non-cancer cell line. Overall, **6a-s** indicated remarkable antioxidant power in scavenging DPPH$^{\cdot}$ and ABTS$^{\cdot+}$; particularly, an analog **6o** with *meta*-methoxy substituent showed most potent radical scavenging activity, whereas scaffolds **6d** with *para*-fluoro, **6k** with *ortho*-methyl, and **6o** with *meta*-methoxy performed excellently in inhibiting both the cervical cancer cell lines and analog **6q** with *meta*-trifluoromethyl substituent expressed excellent sensitivity toward ovarian cancer cell line. From the structure–activity point of view, nature and position of the electron withdrawing and electron donating functional groups on the phenyl ring attached to the tria-

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zole core may contribute to the anticipated antioxidant and anticancer action. Structure of final compounds was adequately confirmed exploring different spectroscopic techniques and elemental analysis in addition to the measurements of some physical properties.

1. Introduction

ROS, reactive oxygen species carrying unpaired electrons are highly reactive molecules, for example superoxide ($O_2^{\bullet-}$), hydroxyl (HO•), peroxyl (ROO•), alkoxyl (RO•), and nitric oxide (NO•) radicals capable of rapidly bind the molecules in adjacent cells. Cellular metabolism processes yield ROS and their access amount results into the damage of essential components of cells such as DNA, lipids and proteins which lead to the occurrence of diseases such as neurological disorders, hypertension, cancer, and diabetes (Weidinger and Kozlov, 2015). This oxidative damage to different kinds of biomolecules is referred to as "oxidative stress". Oxidative stress is regarded to considerably promote the growth of a number of illnesses, particularly age-related diseases (Schieber and Chandel, 2014). Oxidation process is known as a substance response that exchanges hydrogen atom or electrons from a material to an oxidizing agent. The description of antioxidant is initially relevant to elements that avoid the intake of oxygen by human tissues. In other terms, an antioxidant is a compound or varieties that decrease or stops the oxidation of another compound (Göçer et al., 2013).

Among a plenty of natural product verities, polyphenols are the most abundant antioxidants (Scalbert et al., 2005a). Current evidence strongly facilitates a participation of polyphenols to the protection of heart illnesses and malignancies, and indicates a role in the protection of neurodegenerative illnesses and type 2 diabetes (Scalbert et al., 2005b). Hesperidin (3′,5,7-trihydroxy-4′-methoxy-flavanone-7-rhamnoglucoside) is a member of the flavanone group of flavonoids and its deglycosylated forms are known as hesperetin. A number of studies have examined the antioxidant or radical scavenging (Cho, 2006) and anticancer properties (Roohbakhsh et al., 2015; Jeong et al., 1999) of hesperidin and hesperetin. Studies suggested that hesperidin is inactive or only moderately active (Garg et al., 2001; Wilmsen et al., 2005). In contrast, hesperetin was shown to have potent antioxidant effects (Hirata et al., 2005). In addition, reports suggest that hesperetin is a potent inhibitor of the human cervical cancer cell lines (Alshatwi et al., 2013). In a view of enlightened standpoint, we have decided to select hesperetin a as nodule of the rationale design which is connected to the diverse triazole core (Patel and Park, 2014) known to inhibit angiogenesis, a tool for stopping tumor growth and metastasis (Kallander et al., 2005). As cancers figure among the leading causes of fatalities and death rate globally, with approximately 14 million new cases and 8.2 million melanoma related fatalities in 2012 (World Cancer Report, 2014; WHO, 2015) including its direct correlation with ROS, we have decided to prepare new substituted benzyl-1,2,3-triazolyl flavanone derivatives with an aim to obtain newer generation of antioxidant and anticancer agents.

2. Experimental

Commercially available chemicals and solvents were used without purification or after distillation and treatment with drying agents. Hesperidin was purchased from Sigma–Aldrich Company Ltd. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates (Kieselgel 60 F254, Merck) and visualized by UV254 light. Shimadzu 8400-S FT-IR spectrophotometer was used to obtain FT-IR spectra of the title compounds. NMR spectra were taken using a Bruker AVANCE III 400 instrument (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz). ^{1}H NMR spectra are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constant (*J*) in Hertz (Hz). ^{1}H NMR and ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ as internal standard. Purity of compounds was determined by elemental analyses performed using CHN analyzer.

2.1. Synthesis of 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-chroman-4-one (2)

A mixture of **1** (5.7 mmol) and H_2SO_4 (10 mL) in anhydrous CH_3OH (280 mL) was stirred at 60 °C for 9 h, and ethyl acetate (1.2 L) was then added at 20 °C. The solution was washed with H_2O (420 mL), and dried (Na_2SO_4). Evaporation afforded pale-yellow powder. The crude product was dissolved in acetone (70 mL) and added dropwise (60 min) to a stirred mixture of H_2O/acetic acid (150:1, 700 mL) at 95 °C. The slurry was cooled to 45 °C and the product filtered and dried in vacuo to give **2**: Yield: 89%, M.p. 222–224 °C.

2.2. Synthesis of 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(prop-2-ynyloxy)chroman-4-one (4)

Propargyl bromide (**3**) (0.5 mmol) was dropwise added to a solution of compound **2** (1 mmol) in acetonitrile and the reaction mixture was stirred at 75 °C for 4 h. Reaction was monitored by TLC and the crude product was subjected to column chromatography (Hexane: EtOAc) to give pure compound **4**. Yield: 58%. IR (KBr) cm^{-1}: 3365 (OH), 1627 (C═O), 1438 (Ar). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.18 (s, 1H, OH), 9.11 (s, 1H, OH), 6.91–6.86 (m, 3H, Ar—H), 6.38 (d, *J* = 2.2 Hz, 1H), 6.21 (d, *J* = 2.3 Hz, 1H), 5.47 (dd, *J* = 2.6, 12.2 Hz, 1H), 5.22 (s, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.19 (dd, *J* = 12.7, 17.2 Hz,1H, 3-H trans), 2.61 (dd, *J* = 2.8, 17.2 Hz, 1H, 3-H cis), 2.29 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 186.4, 165.7, 163.3, 161.5, 157.6, 154.2, 135.1, 119.0, 111.9, 110.1, 102.4, 98.5, 93.8, 84.2, 80.3, 78.7, 66.5 (OCH_3), 64.4 (OCH_2), 45.6. EMI–MS (*m*/*z*): 341.69 (M^+). Anal. Calcd. for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74. Found: C, 67.22; H, 4.59.

*2.3. General procedure for the synthesis of compounds **6a-6s***

Appropriate benzyl derivatives (0.65 mmol) were sonicated at 35 °C in the presence of a solution of NaN_3 (0.8 mmol) in DMF (15 mL) and the progress of the reaction was monitored by TLC. After completion, $CuSO_4{\cdot}5H_2O$ (0.12 mmol) and sodium ascorbate (0.30 mmol) as well as intermediate **4** (0.5 mmol) were added and reaction was allowed to stir at room temperature for another 3 h. After completion, mixture was filtered, and solvent was evaporated under reduced pressure. The crude thus obtained was extracted with dichloromethane (3×40 mL) and the combined organic layer was dried over sodium sulfate and purified through column chromatography (MeOH:$CHCl_3$) to afford the final product **6a-6s** in 40–85% yield.

*2.3.1. 7-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6a**)*

Yield: 53%. M.p. 189–191 °C. IR (KBr) cm^{-1}: 3391 (OH), 1644 (C═O), 1560 (C═C, triazole), 1466 (C—N, triazole), 1450 (Ar), 1403 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.12 (s, 1H, OH), 9.18 (s, 1H, OH), 7.62 (s, 1H, triazole-H), 7.41–7.34 (m, 5H, Ar—H), 6.95–6.87 (m, 3H, Ar—H), 6.30 (d, J = 2.2 Hz, 1H), 6.18 (d, J = 2.3 Hz, 1H), 5.51 (dd, J = 2.6, 12.2 Hz, 1H), 5.29 (s, 2H, CH_2), 5.17 (s, 2H, OCH_2), 3.77 (s, 3H, OCH_3), 3.21 (dd, J = 12.7, 17.2 Hz,1H, 3-H trans), 2.65 (dd, J = 2.8, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.2, 180.9, 165.3, 163.1, 161.6, 160.6, 157.4, 154.8, 146.2 (C-4, triazole), 141.5, 137.1, 135.3, 132.9 (C-5, triazole), 130.7, 129.1, 125.4, 124.6, 123.8, 119.9, 110.1, 106.5, 98.2, 93.8, 66.2 (OCH_3), 64.3 (OCH_2), 54.6 (N—CH_2). EMI–MS (m/z): 474.67 (M^+). Anal. Calcd. for $C_{26}H_{23}N_3O_6$: C, 65.95; H, 4.90; N, 8.87. Found: C, 66.03; H, 4.98; N, 8.96.

*2.3.2. 7-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6b**)*

Yield: 44%. M.p. 161–163 °C. IR (KBr) cm^{-1}: 3360 (OH), 1650 (C═O), 1553 (C═C, triazole), 1472 (C—N, triazole), 1444 (Ar), 1409 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.06 (s, 1H, OH), 9.12 (s, 1H, OH), 7.56 (s, 1H, triazole-H), 7.44–7.31 (m, 4H, Ar—H), 6.97–6.89 (m, 3H, Ar—H), 6.42 (d, J = 2.3 Hz, 1H), 6.18 (d, J = 2.2 Hz, 1H), 5.39 (dd, J = 2.7, 12.4 Hz, 1H), 5.23 (s, 2H, CH_2), 5.11 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3), 3.15 (dd, J = 12.6, 17.1 Hz,1H, 3-H trans), 2.71 (dd, J = 2.9, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.1, 179.2, 166.8, 164.6, 162.0, 159.7, 156.2, 153.0, 145.8 (C-4, triazole), 142.6, 138.5, 136.8, 132.7 (C-5, triazole), 131.2, 128.0, 126.8, 125.1, 124.5, 118.2, 111.8, 107.4, 98.6, 94.9, 67.1 (OCH_3), 65.0 (OCH_2), 55.1 (N—CH_2). EMI–MS (m/z): 492.29 (M^+). Anal. Calcd. for $C_{26}H_{22}FN_3O_6$: C, 63.54; H, 4.51; N, 8.55. Found: C, 63.67; H, 4.37; N, 8.42.

*2.3.3. 7-((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6c**)*

Yield: 41%. M.p. 173–175 °C. IR (KBr) cm^{-1}: 3402 (OH), 1636 (C═O), 1567 (C═C, triazole), 1459 (C—N, triazole), 1437 (Ar), 1415 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.18 (s, 1H, OH), 9.06 (s, 1H, OH), 7.68 (s, 1H, triazole-H), 7.42–7.33 (m, 4H, Ar—H), 6.94–6.86 (m, 3H, Ar—H), 6.36 (d, J = 2.4 Hz, 1H), 6.24 (d, J = 2.4 Hz, 1H), 5.45 (dd, J = 2.5, 12.3 Hz, 1H), 5.36 (s, 2H, CH_2), 5.06 (s, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.27 (dd, J = 12.5, 17.3 Hz,1H, 3-H trans), 2.77 (dd, J = 2.7, 17.1 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.4, 180.7, 166.6, 163.3, 162.8, 160.4, 157.6, 153.1, 146.4 (C-4, triazole), 142.8, 137.3, 135.9, 133.0 (C-5, triazole), 130.5, 128.8, 125.6, 124.8, 123.6, 118.4, 110.3, 107.6, 99.8, 93.6, 66.4 (OCH_3), 64.8 (OCH_2), 54.4 (N—CH_2). EMI–MS (m/z): 492.63 (M^+). Anal. Calcd. for $C_{26}H_{22}FN_3O_6$: C, 63.54; H, 4.51; N, 8.55. Found: C, 63.69; H, 4.63; N, 8.45.

*2.3.4. 7-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6d**)*

Yield: 49%. M.p. 182–184 °C. IR (KBr) cm^{-1}: 3380 (OH), 1645 (C═O), 1561 (C═C, triazole), 1467 (C—N, triazole), 1451 (Ar), 1404 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.13 (s, 1H, OH), 9.19 (s, 1H, OH), 7.63 (s, 1H, triazole-H), 7.40–7.30 (m, 4H, Ar—H), 6.96–6.85 (m, 3H, Ar—H), 6.31 (d, J = 2.4 Hz, 1H), 6.19 (d, J = 2.2 Hz, 1H), 5.52 (dd, J = 2.5, 12.4 Hz, 1H), 5.30 (s, 2H, CH_2), 5.18 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 3.22 (dd, J = 12.6, 17.3 Hz,1H, 3-H trans), 2.66 (dd, J = 2.7, 17.1 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.3, 179.4, 165.5, 164.8, 161.4, 159.5, 156.4, 154.6, 145.1 (C-4, triazole), 141.9, 138.7, 136.4, 133.5 (C-5, triazole), 131.4, 129.3, 126.6, 125.3, 124.3, 119.7, 111.6, 106.7, 99.4, 94.7, 67.3 (OCH_3), 65.2 (OCH_2), 55.0 (N—CH_2). EMI–MS (m/z): 492.75 (M^+). Anal. Calcd. for $C_{26}H_{22}FN_3O_6$: C, 63.54; H, 4.51; N, 8.55. Found: C, 63.41; H, 4.39; N, 8.40.

*2.3.5. 7-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6e**)*

Yield: 51%. M.p. 176–178 °C. IR (KBr) cm^{-1}: 3356 (OH), 1651 (C═O), 1554 (C═C, triazole), 1473 (C—N, triazole), 1445 (Ar), 1410 (N═N, triazole), 750 (C—Cl). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.07 (s, 1H, OH), 9.13 (s, 1H, OH), 7.57 (s, 1H, triazole-H), 7.43–7.32 (m, 4H, Ar—H), 6.98–6.90 (m, 3H, Ar—H), 6.43 (d, J = 2.3 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 5.40 (dd, J = 2.6, 12.3 Hz, 1H), 5.24 (s, 2H, CH_2), 5.12 (s, 2H, OCH_2), 3.72 (s, 3H, OCH_3), 3.16 (dd, J = 12.7, 17.2 Hz,1H, 3-H trans), 2.72 (dd, J = 2.9, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.7, 180.5, 165.7, 163.5, 161.2, 160.2, 157.8, 154.4, 146.6 (C-4, triazole), 141.7, 138.9, 135.5, 132.3 (C-5, triazole), 130.3, 129.5, 125.8, 124.0, 123.4, 119.5, 110.5, 106.9, 98.8, 93.4, 66.8 (OCH_3), 64.1 (OCH_2), 55.3 (N—CH_2). EMI–MS (m/z): 508.78 (M^+). Anal. Calcd. for $C_{26}H_{22}ClN_3O_6$: C, 61.48; H, 4.37; N, 8.27. Found: C, 61.36; H, 4.51; N, 8.40.

*2.3.6. 7-((1-(3-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6f**)*

Yield: 43%. M.p. 193–195 °C. IR (KBr) cm^{-1}: 3410 (OH), 1637 (C═O), 1568 (C═C, triazole), 1460 (C—N, triazole), 1438 (Ar), 1416 (N═N, triazole), 765 (C—Cl). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.19 (s, 1H, OH), 9.07 (s, 1H, OH), 7.69 (s, 1H, triazole-H), 7.45–7.35 (m, 4H, Ar—H), 6.93–6.88 (m, 3H, Ar—H), 6.37 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 2.4 Hz, 1H), 5.46 (dd, J = 2.7, 12.2 Hz, 1H), 5.37 (s, 2H, CH_2), 5.07 (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.28 (dd,

J = 12.5, 17.1 Hz,1H, 3-H trans), 2.78 (dd, J = 2.8, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.6, 179.6, 166.4, 164.4, 162.6, 160.0, 156.6, 153.3, 145.3 (C-4, triazole), 142.1, 137.5, 136.2, 132.5 (C-5, triazole), 131.6, 128.6, 126.4, 125.5, 124.7, 118.6, 111.4, 107.8, 98.4, 94.5, 67.5 (OCH_3), 65.4 (OCH_2), 54.2 (N—CH_2). EMI–MS (m/z): 508.72 (M^+). Anal. Calcd. for $C_{26}H_{22}ClN_3O_6$: C, 61.48; H, 4.37; N, 8.27. Found: C, 61.35; H, 4.29; N, 8.38.

*2.3.7. 7-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6g**)*

Yield: 52%. M.p. 165–167 °C. IR (KBr) cm^{-1}: 3376 (OH), 1646 (C═O), 1562 (C═C, triazole), 1468 (C—N, triazole), 1452 (Ar), 1405 (N═N, triazole), 778 (C—Cl). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.14 (s, 1H, OH), 9.20 (s, 1H, OH), 7.64 (s, 1H, triazole-H), 7.41–7.31 (m, 4H, Ar—H), 6.95–6.89 (m, 3H, Ar—H), 6.32 (d, J = 2.2 Hz, 1H), 6.20 (d, J = 2.4 Hz, 1H), 5.53 (dd, J = 2.7, 12.2 Hz, 1H), 5.31 (s, 2H, CH_2), 5.19 (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3), 3.23 (dd, J = 12.5, 17.1 Hz,1H, 3-H trans), 2.67 (dd, J = 2.8, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.1, 179.8, 166.2, 163.7, 162.4, 159.3, 157.2, 153.5, 146.8 (C-4, triazole), 142.3, 137.7, 135.7, 132.7 (C-5, triazole), 130.1, 128.4, 125.0, 124.2, 123.2, 118.8, 110.7, 107.2, 99.6, 93.2, 66.9 (OCH_3), 64.7 (OCH_2), 54.1 (N—CH_2). EMI–MS (m/z): 508.96 (M^+). Anal. Calcd. for $C_{26}H_{22}ClN_3O_6$: C, 61.48; H, 4.37; N, 8.27. Found: C, 61.62; H, 4.32; N, 8.34.

*2.3.8. 7-((1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6h**)*

Yield: 47%. M.p. 156–158 °C. IR (KBr) cm^{-1}: 3362 (OH), 1652 (C═O), 1555 (C═C, triazole), 1474 (C—N, triazole), 1446 (Ar), 1411 (N═N, triazole). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.08 (s, 1H, OH), 9.14 (s, 1H, OH), 7.58 (s, 1H, triazole-H), 7.44–7.33 (m, 4H, Ar—H), 6.97–6.86 (m, 3H, Ar—H), 6.44 (d, J = 2.4 Hz, 1H), 6.14 (d, J = 2.3 Hz, 1H), 5.41 (dd, J = 2.5, 12.4 Hz, 1H), 5.25 (s, 2H, CH_2), 5.13 (s, 2H, OCH_2), 3.73 (s, 3H, OCH_3), 3.17 (dd, J = 12.6, 17.3 Hz,1H, 3-H trans), 2.73 (dd, J = 2.7, 17.1 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.5, 180.3, 165.9, 164.2, 161.0, 159.1, 156.8, 154.2, 145.5 (C-4, triazole), 141.8, 138.2, 136.9, 133.9 (C-5, triazole), 131.8, 129.7, 126.2, 125.7, 124.9, 119.3, 111.2, 106.1, 98.0, 94.3, 67.7 (OCH_3), 65.6 (OCH_2), 55.5 (N—CH_2). EMI–MS (m/z): 553.55 (M^+). Anal. Calcd. for $C_{26}H_{22}BrN_3O_6$: C, 56.53; H, 4.01; N, 7.61. Found: C, 56.38; H, 4.13; N, 7.50.

*2.3.9. 7-((1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6i**)*

Yield: 42%. M.p. 186–188 °C. IR (KBr) cm^{-1}: 3415 (OH), 1638 (C═O), 1569 (C═C, triazole), 1461 (C—N, triazole), 1439 (Ar), 1417 (N═N, triazole). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.20 (s, 1H, OH), 9.08 (s, 1H, OH), 7.70 (s, 1H, triazole-H), 7.42–7.34 (m, 4H, Ar—H), 6.94–6.85 (m, 3H, Ar—H), 6.38 (d, J = 2.3 Hz, 1H), 6.26 (d, J = 2.2 Hz, 1H), 5.47 (dd, J = 2.6, 12.3 Hz, 1H), 5.38 (s, 2H, CH_2), 5.08 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.29 (dd, J = 12.7, 17.2 Hz,1H, 3-H trans), 2.79 (dd, J = 2.9, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.8, 180.1, 165.1, 163.9, 161.1, 160.9, 157.0, 154.9, 146.1 (C-4, triazole), 141.6, 137.4, 135.1, 132.1 (C-5, triazole), 130.9, 129.2, 125.1, 124.4, 123.9, 119.1, 110.9, 106.3, 99.2, 93.0, 66.6 (OCH_3), 64.9 (OCH_2), 55.7 (N—CH_2). EMI–MS (m/z): 553.23 (M^+). Anal. Calcd. for $C_{26}H_{22}BrN_3O_6$: C, 56.53; H, 4.01; N, 7.61. Found: C, 56.65; H, 4.14; N, 7.70.

*2.3.10. 7-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one (**6j**)*

Yield: 50%. M.p. 175–177 °C. IR (KBr) cm^{-1}: 3394 (OH), 1647 (C═O), 1563 (C═C, triazole), 1469 (C—N, triazole), 1453 (Ar), 1406 (N═N, triazole). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.15 (s, 1H, OH), 9.21 (s, 1H, OH), 7.65 (s, 1H, triazole-H), 7.40–7.32 (m, 4H, Ar—H), 6.96–6.87 (m, 3H, Ar—H), 6.33 (d, J = 2.3 Hz, 1H), 6.21 (d, J = 2.2 Hz, 1H), 5.54 (dd, J = 2.6, 12.3 Hz, 1H), 5.32 (s, 2H, CH_2), 5.20 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 3.24 (dd, J = 12.7, 17.2 Hz,1H, 3-H trans), 2.68 (dd, J = 2.9, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.2, 179.9, 166.7, 164.0, 162.2, 159.0, 156.9, 153.7, 145.7 (C-4, triazole), 142.5, 137.9, 135.8, 132.8 (C-5, triazole), 131.1, 128.9, 126.9, 125.2, 124.8, 118.9, 111.1, 107.0, 98.9, 94.1, 67.9 (OCH_3), 65.8 (OCH_2), 54.3 (N—CH_2). EMI–MS (m/z): 553.62 (M^+). Anal. Calcd. for $C_{26}H_{22}BrN_3O_6$: C, 56.53; H, 4.01; N, 7.61. Found: C, 56.61; H, 4.09; N, 7.52.

*2.3.11. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)chroman-4-one (**6k**)*

Yield: 46%. M.p. 160–162 °C. IR (KBr) cm^{-1}: 3353 (OH), 1653 (C═O), 1556 (C═C, triazole), 1475 (C—N, triazole), 1447 (Ar), 1412 (N═N, triazole). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.09 (s, 1H, OH), 9.15 (s, 1H, OH), 7.59 (s, 1H, triazole-H), 7.43–7.30 (m, 4H, Ar—H), 6.98–6.88 (m, 3H, Ar—H), 6.45 (d, J = 2.4 Hz, 1H), 6.15 (d, J = 2.4 Hz, 1H), 5.42 (dd, J = 2.7, 12.2 Hz, 1H), 5.26 (s, 2H, CH_2), 5.14 (s, 2H, OCH_2), 3.74 (s, 3H, OCH_3), 3.18 (dd, J = 12.5, 17.3 Hz,1H, 3-H trans), 2.74 (dd, J = 2.8, 17.1 Hz, 1H, 3-H cis), 1.94 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.4, 180.0, 165.0, 163.2, 161.3, 160.7, 157.1, 153.9, 146.3 (C-4, triazole), 141.4, 137.2, 136.6, 133.2 (C-5, triazole), 130.8, 128.2, 125.3, 124.9, 123.7, 118.7, 110.2, 107.1, 99.1, 93.9, 67.0 (OCH_3), 64.5 (OCH_2), 54.4 (N—CH_2), 20.4. EMI–MS (m/z): 488.27 (M^+). Anal. Calcd. for $C_{27}H_{25}N_3O_6$: C, 66.52; H, 5.17; N, 8.62. Found: C, 66.65; H, 5.25; N, 8.50.

*2.3.12. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)chroman-4-one (**6l**)*

Yield: 48%. M.p. 153–155 °C. IR (KBr) cm^{-1}: 3408 (OH), 1639 (C═O), 1570 (C═C, triazole), 1462 (C—N, triazole), 1440 (Ar), 1418 (N═N, triazole). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.21 (s, 1H, OH), 9.09 (s, 1H, OH), 7.71 (s, 1H, triazole-H), 7.45–7.36 (m, 4H, Ar—H), 6.93–6.84 (m, 3H, Ar—H), 6.36 (d, J = 2.2 Hz, 1H), 6.27 (d, J = 2.3 Hz, 1H), 5.48 (dd, J = 2.5, 12.3 Hz, 1H), 5.30 (s, 2H, CH_2), 5.09 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.30 (dd, J = 12.6, 17.1 Hz,1H, 3-H trans), 2.80 (dd, J = 2.7, 17.3 Hz, 1H, 3-H cis), 1.83 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.0, 179.7, 166.9, 164.1, 162.9, 160.5, 156.7, 154.7, 145.0 (C-4, triazole), 141.2, 138.6, 136.7, 132.6 (C-5, triazole), 130.6, 129.4, 125.5, 124.7, 123.5, 119.0, 111.5, 106.2, 98.7,

94.2, 66.7 (OCH_3), 65.1 (OCH_2), 55.9 (N—CH_2), 22.7. EMI–MS (*m*/*z*): 488.67 (M^+). Anal. Calcd. for $C_{27}H_{25}N_3O_6$: C, 66.52; H, 5.17; N, 8.62. Found: C, 66.61; H, 5.08; N, 8.70.

*2.3.13. 2-((4-((5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl) benzonitrile (**6m**)*

Yield: 40%. M.p. 185–187 °C. IR (KBr) cm^{-1}: 3367 (OH), 1648 (C═O), 1564 (C═C, triazole), 1470 (C—N, triazole), 1454 (Ar), 1407 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.16 (s, 1H, OH), 9.22 (s, 1H, OH), 7.66 (s, 1H, triazole-H), 7.41–7.32 (m, 4H, Ar—H), 6.95–6.88 (m, 3H, Ar—H), 6.34 (d, *J* = 2.3 Hz, 1H), 6.22 (d, *J* = 2.3 Hz, 1H), 5.55 (dd, *J* = 2.6, 12.4 Hz, 1H), 5.33 (s, 2H, CH_2), 5.21 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 3.25 (dd, *J* = 12.6, 17.1 Hz,1H, 3-H trans), 2.69 (dd, *J* = 2.8, 17.1 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.9, 180.2, 165.2, 163.4, 162.7, 159.8, 157.3, 154.5, 146.5 (C-4, triazole), 142.7, 138.8, 135.6, 133.8 (C-5, triazole), 131.3, 128.5, 126.7, 125.9, 124.0, 119.8, 117.3, 110.4, 106.4, 99.3, 93.7, 66.5 (OCH_3), 65.3 (OCH_2), 55.2 (N—CH_2). EMI–MS (*m*/*z*): 499.31 (M^+). Anal. Calcd. for $C_{27}H_{22}N_4O_6$: C, 65.05; H, 4.45; N, 11.24. Found: C, 64.97; H, 4.33; N, 11.12.

*2.3.14. 4-((4-((5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl) benzonitrile (**6n**)*

Yield: 43%. M.p. 178–180 °C. IR (KBr) cm^{-1}: 3374 (OH), 1654 (C═O), 1557 (C═C, triazole), 1476 (C—N, triazole), 1448 (Ar), 1413 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.10 (s, 1H, OH), 9.16 (s, 1H, OH), 7.60 (s, 1H, triazole-H), 7.44–7.35 (m, 4H, Ar—H), 6.97–6.87 (m, 3H, Ar—H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.16 (d, *J* = 2.2 Hz, 1H), 5.43 (dd, *J* = 2.7, 12.4 Hz, 1H), 5.27 (s, 2H, CH_2), 5.15 (s, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 3.19 (dd, *J* = 12.7, 17.2 Hz,1H, 3-H trans), 2.75 (dd, *J* = 2.9, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.6, 179.5, 166.5, 164.3, 161.5, 159.6, 156.0, 153.2, 145.2 (C-4, triazole), 142.9, 137.4, 135.2, 132.4 (C-5, triazole), 131.5, 129.6, 126.5, 125.4, 124.6, 118.5, 119.7, 111.3, 107.3, 98.5, 94.4, 67.8 (OCH_3), 64.6 (OCH_2), 54.5 (N—CH_2). EMI–MS (*m*/*z*): 499.62 (M^+). Anal. Calcd. for $C_{27}H_{22}N_4O_6$: C, 65.05; H, 4.45; N, 11.24. Found: C, 64.98; H, 4.54; N, 11.33.

*2.3.15. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy) chroman-4-one (**6o**)*

Yield: 50%. M.p. 164–166 °C. IR (KBr) cm^{-1}: 3405 (OH), 1640 (C═O), 1571 (C═C, triazole), 1463 (C—N, triazole), 1441 (Ar), 1419 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.22 (s, 1H, OH), 9.10 (s, 1H, OH), 7.72 (s, 1H, triazole-H), 7.42–7.31 (m, 4H, Ar—H), 6.94–6.88 (m, 3H, Ar—H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 5.49 (dd, *J* = 2.5, 12.3 Hz, 1H), 5.31 (s, 2H, CH_2), 5.10 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.31 (dd, *J* = 12.5, 17.3 Hz,1H, 3-H trans), 2.81 (dd, *J* = 2.7, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.7, 179.3, 165.4, 163.6, 162.5, 160.3, 157.5, 153.4, 146.7 (C-4, triazole), 141.1, 138.0, 136.5, 133.6 (C-5, triazole), 130.4, 128.3, 125.7, 124.5, 123.3, 119.6, 110.6, 107.5, 99.9, 93.5, 67.6 (OCH_3), 66.2, 64.4 (OCH_2), 55.4 (N—CH_2). EMI–MS (*m*/*z*): 504.66 (M^+). Anal. Calcd. for $C_{27}H_{25}N_3O_7$: C, 64.41; H, 5.00; N, 8.35. Found: C, 64.30; H, 4.88; N, 8.47.

*2.3.16. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)chroman-4-one (**6p**)*

Yield: 53%. M.p. 157–159 °C. IR (KBr) cm^{-1}: 3359 (OH), 1641 (C═O), 1565 (C═C, triazole), 1471 (C—N, triazole), 1455 (Ar), 1408 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.17 (s, 1H, OH), 9.23 (s, 1H, OH), 7.67 (s, 1H, triazole-H), 7.40–7.33 (m, 4H, Ar—H), 6.96–6.86 (m, 3H, Ar—H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.23 (d, *J* = 2.3 Hz, 1H), 5.56 (dd, *J* = 2.7, 12.2 Hz, 1H), 5.34 (s, 2H, CH_2), 5.22 (s, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.26 (dd, *J* = 12.6, 17.2 Hz,1H, 3-H trans), 2.70 (dd, *J* = 2.8, 17.2 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.0, 180.4, 163.3, 164.5, 161.7, 159.4, 156.3, 154.3, 145.4 (C-4, triazole), 142.0, 137.6, 136.3, 132.2 (C-5, triazole), 130.2, 129.8, 125.9, 124.3, 123.1, 118.3, 111.9, 106.6, 98.3, 94.6, 66.3 (OCH_3), 65.9, 65.5 (OCH_2), 54.7 (N—CH_2). EMI–MS (*m*/*z*): 504.36 (M^+). Anal. Calcd. for $C_{27}H_{25}N_3O_7$: C, 64.41; H, 5.00; N, 8.35. Found: C, 64.32; H, 5.09; N, 8.27.

*2.3.17. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(3-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy) chroman-4-one (**6q**)*

Yield: 44%. M.p. 194–196 °C. IR (KBr) cm^{-1}: 3420 (OH), 1655 (C═O), 1558 (C═C, triazole), 1477 (C—N, triazole), 1449 (Ar), 1414 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.11 (s, 1H, OH), 9.17 (s, 1H, OH), 7.61 (s, 1H, triazole-H), 7.43–7.34 (m, 4H, Ar—H), 6.98–6.89 (m, 3H, Ar—H), 6.47 (d, *J* = 2.2 Hz, 1H), 6.17 (d, *J* = 2.4 Hz, 1H), 5.44 (dd, *J* = 2.6, 12.4 Hz, 1H), 5.28 (s, 2H, CH_2), 5.16 (s, 2H, OCH_2), 3.76 (s, 3H, OCH_3), 3.20 (dd, *J* = 12.7, 17.1 Hz,1H, 3-H trans), 2.76 (dd, *J* = 2.9, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.5, 180.8, 165.6, 163.8, 162.3, 160.1, 157.7, 153.6, 146.9 (C-4, triazole), 142.2, 138.1, 135.4, 133.4 (C-5, triazole), 131.7, 128.7, 126.3, 125.6, 124.9, 124.4, 119.4, 110.8, 106.0, 99.5, 93.3, 66.1 (OCH_3), 65.7 (OCH_2), 55.6 (N—CH_2). EMI–MS (*m*/*z*): 541.32 (M^+). Anal. Calcd. for $C_{27}H_{22}F_3N_3O_6$: C, 59.89; H, 4.10; N, 7.76. Found: C, 59.96; H, 4.23; N, 7.89.

*2.3.18. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(4-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)methoxy) chroman-4-one (**6r**)*

Yield: 43%. M.p. 190–192 °C. IR (KBr) cm^{-1}: 3411 (OH), 1642 (C═O), 1572 (C═C, triazole), 1464 (C—N, triazole), 1442 (Ar), 1420 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.23 (s, 1H, OH), 9.11 (s, 1H, OH), 7.73 (s, 1H, triazole-H), 7.45–7.32 (m, 4H, Ar—H), 6.93–6.83 (m, 3H, Ar—H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.29 (d, *J* = 2.2 Hz, 1H), 5.50 (dd, *J* = 2.5, 12.3 Hz, 1H), 5.32 (s, 2H, CH_2), 5.05 (s, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 3.32 (dd, *J* = 12.6, 17.3 Hz,1H, 3-H trans), 2.82 (dd, *J* = 2.8, 17.1 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.8, 179.1, 166.0, 164.7, 161.9, 159.2, 156.1, 154.1, 145.6 (C-4, triazole), 141.3, 137.8, 135.0, 132.0 (C-5, triazole), 131.9, 129.0, 126.1, 125.8, 125.3, 124.2, 118.1, 111.7, 107.7, 98.1, 94.8, 67.4 (OCH_3), 64.2 (OCH_2), 54.9 (N—CH_2). EMI–MS (*m*/*z*): 541.68 (M^+). Anal. Calcd. for $C_{27}H_{22}F_3N_3O_6$: C, 59.89; H, 4.10; N, 7.76. Found: C, 59.77; H, 4.02; N, 7.64.

*2.3.19. 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)chroman-4-one (**6s**)*

Yield: 45%. M.p. 177–179 °C. IR (KBr) cm^{-1}: 3389 (OH), 1642 (C═O), 1566 (C═C, triazole), 1465 (C—N, triazole), 1443 (Ar), 1407 (N═N, triazole). ^1H NMR ($CDCl_3$, 400 MHz): δ 12.07 (s, 1H, OH), 9.24 (s, 1H, OH), 7.63 (s, 1H, triazole-H), 7.43–7.35 (m, 4H, Ar—H), 6.95–6.86 (m, 3H, Ar—H), 6.48 (d, J = 2.4 Hz, 1H), 6.19 (d, J = 2.3 Hz, 1H), 5.57 (dd, J = 2.7, 12.4 Hz, 1H), 5.35 (s, 2H, CH_2), 5.23 (s, 2H, OCH_2), 3.71 (s, 3H, OCH_3), 3.21 (dd, J = 12.5, 17.2 Hz,1H, 3-H trans), 2.72 (dd, J = 2.7, 17.3 Hz, 1H, 3-H cis). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.3, 180.6, 165.8, 163.0, 162.1, 160.8, 157.9, 153.8, 146.0 (C-4, triazole), 142.4, 138.3, 136.1, 133.1 (C-5, triazole), 130.0, 128.0, 125.2, 124.1, 123.0, 119.2, 110.0, 106.8, 99.7, 93.1, 67.2 (OCH_3), 65.9 (OCH_2), 55.8 (N—CH_2). EMI–MS (m/z): 519.60 (M^+). Anal. Calcd. for $C_{26}H_{22}N_4O_8$: C, 60.23; H, 4.28; N, 10.81. Found: C, 60.36; H, 4.15; N, 10.92.

2.4. Biological assays

2.4.1. DPPH free radical scavenging assay

Free radicals exercise deleterious role in biological systems and in foods and hence radical scavenging activities are very useful. Various chemical reactions running in the biological systems usually furnish free radicals which are responsible to cause damage to the building block of biologics such as DNA and lipids. Reduction of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl is the base of DPPH antioxidant bioassay. It has an odd electron which exerts a maximum absorption band of 517 nm (deep violet color) in ethanol. The DPPH bioassay is the widely used and acceptable method for inspecting the free radical scavenging efficacy of the intended compound. Such substances donate a hydrogen atom when it mixes with the DPPH thereby introducing its reduced congener, diphenylpicrylhydrazine (nonradical) with the loss of violet color.

In the present study, DPPH bioassay was adopted to screen **6a-s** for their *in vitro* antioxidant potencies. The results of this bioassay screenings were presented in the form of the percentage of radical scavenging antioxidant activity (RSA%) of each substance. The investigation of the DPPH radical scavenging activity was operated according to the methodology described by Brand-Williams et al. (1995). A stable free radical, 2,2-diphenyl-1-picrylhydrazyl was allowed to react with **6a-s** in methanol solvent as 20 μL quantities of titled compounds were mixed up with 180 μL of DPPH in MeOH. These titled compounds donated hydrogen in this mixing thereby carried out reduction of DPPH and hence a change in the color was observed from deep violet to light yellow at 517 nm after 25 min of reaction using a UV–Visible spectrophotometer (Perkin Elmer). The lank reading was also performed using the mixture of methanol (20 μL) and sample (180 μL of DPPH). Ascorbic acid served as a control drug in this assay and its solution was prepared upon mixing methanol (20 μL) and DPPH radical solution (180 μL). The results of this bioassay, RSA% (the radical scavenging activity in percentage) were determined according to Mensor et al. (2001) as described in below equation.

$$\%\ Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

A plot between concentration of test compounds and % scavenging introduced IC_{50} levels in the presence of Ascorbic acid as standard.

2.4.2. ABTS radical scavenging assay

The $ABTS^{\cdot+}$ radical cation scavenging efficacies of the test compounds were determined according to the method described earlier (Re et al., 1999). Mixing of an equal amount of 7 mM $ABTS^{\cdot+}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) stock solution with 2.45 mM potassium persulfate stock solution produces the $ABTS^{\cdot+}$ cation. The mixture was kept in dark place at 0 °C temperature for 12 h and ABTS solution was diluted with MeOH so that it gives UV absorption value of 0.700 (±0.200) at the 734 nm. The 1000 μL stock solutions of titled compounds 3**a-j** were established upon dissolving them in MeOH and further dilutions furnish 100 μL, 10 μL, 1 μL, and 0.1 μL of quantities of samples. 180 μL solutions of compounds to be evaluated and 20 μL of the ABTS solution was mixed in 96 well plates in dark place which were then incubated for 10 min to measure UV absorption at 734 nm. Mixture of 180 μL ABTS and 20 μL mL methanol was used as a control determination, whereas ascorbic acid was used as a reference drug. The UV absorption data represented the radical scavenging rates which give the corresponding IC_{50} levels for the test compounds.

The scavenging capability of $ABTS^{\cdot+}$ radical was calculated using the following equation:

$$\%\ Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

2.5. In vitro anticancer bioassay

The test compounds **6a-s** were checked for their *in vitro* anticancer potential against cervical cancer cell lines (HeLa and CaSki) and an ovarian cancer cell line (SK-OV-3) and cytotoxic action against Madin Darby canine kidney (MDCK) cells which were purchased from American Type Culture Collection (ATCC). All the cell lines were well maintained in a humidified cell culture incubator in the presence of 5% of CO_2 at 32 °C temperature. Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 Medium supplemented with 10% of fetal Bovine Serum (FBS) and 1% of Antibiotic–Antimycotic solution (100×) were used for HeLa, CaSki, SK-OV-3 and MDCK cell growth respectively. DMEM, RPMI-1640, trypsin–EDTA, Antibiotic–Antimycotic Solution 100× and FBS were purchased from Welgene (150-Seongseo Industrial complex Bukro, Dalseogu, Daegu, 704–948 Republic of Korea).

In the 96 well plates, all cancer cell lines (HeLa, CaSki and SK-OV-3) and a non-cancer cell line (MDCK) were seeded and plates were concentrated as 2×10^4 cells per well plate. Cancerous cells were allowed to grow for 1 day initially and after that the 96 well plates were washed twice with phosphate buffer saline (PBS). DMEM and RPMI-1640 medium contained trypsin–EDTA were used to dilute HeLa, CaSki, SK-OV-3 and MDCK cells up to 5×10^3 level which was used for the infection followed by placing of 10 μL of compound quantities and 90 μL of cell solution onto the 96 well plates in which HeLa, CaSki, SK-OV-3 and MDCK cells were

grown the previous day. 0.1 μL, 1 μL, 10 μL and 100 μL concentrations of the test compounds were used in 96 well plates for the analysis with three replicates of observations. Infected plates were incubated in CO_2 incubator for a period of 48 h. After incubation the medium was removed and washed twice with PBS buffer. After that, 70% of acetone was added to fix the cells and was incubated for 1 h at 4 °C temperature. After incubation, solvent was removed and plates were dried in an oven at 60 °C temperature. The dried plates were overnight incubated with 100 μL of SRB (0.4 mg/L) followed by SRB removal and washing thrice with 1% of acetic acid and dried again under hot air oven at 60 °C. Microscopic observation was carried out to determine the morphology of the cells and after this observation the SRB strain was dissolved with 10 mM of Tris base and incubated overnight (Adaramoye et al., 2011; Mistry et al., 2015). Spectrophotometric data were recorded at 510 nm to calculate the inhibition concentration of 50% (IC_{50}) and cytotoxic concentration of 50% (CC_{50}).

3. Results and discussion

3.1. Chemistry

Titled compounds **6a-s** were synthesized via efficient reaction sequences as described in Scheme 1. Hesperidin (**1**) was subjected to hydrolysis in the presence of sulfuric acid in methanol to give hesperetin **2** (Seitz and Wingard, 1987). Nucleophilic substitution reaction of **2** with propargyl bromide (**3**) yielded intermediate **4**. Desired benzyl halides were diazotized in acidic conditions and then treated with sodium azide to construct corresponding azides (**5a-s**) Jin et al., 2014. $CuSO_4{\cdot}5H_2O$ and sodium ascorbate in DMF were utilized for the azide–alkyne cycloaddition through click chemistry between aromatic azides (**5a-s**) and intermediate **4** to furnish final 1,2,3-triazole based hesperetin analogs **6a-s** in reasonably good yields. Structural elucidation using ^1H NMR, ^{13}C NMR and mass spectrometry was in good accordance with assumed structures. Newer compounds gave C, H and N analyses within 0.4% points from the theoretical values, i.e. in acceptable range.

Table 1 Screening results of DPPH and ABTS radical scavenging activity of **6a-s**.

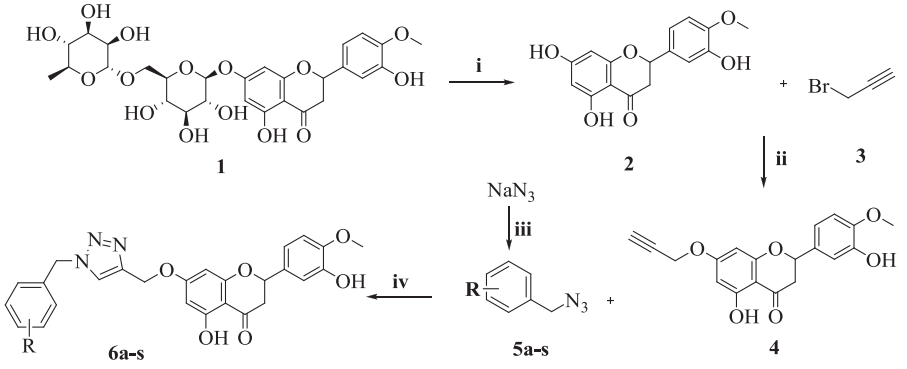
No.	R	IC_{50} μM ± SD[a]	
		DPPH	ABTS
6a	H	56.45 ± 2.339	8.545 ± 0.545
6b	2-F	52.12 ± 1.568	12.645 ± 0.733
6c	3-F	44.96 ± 2.034	15.645 ± 2.280
6d	4-F	37.34 ± 3.397	13.534 ± 0.471
6e	2-Cl	43.85 ± 1.645	17.532 ± 1.325
6f	3-Cl	71.27 ± 1.143	19.542 ± 0.843
6g	4-Cl	41.90 ± 0.754	33.582 ± 2.422
6h	2-Br	41.63 ± 1.533	16.608 ± 0.356
6i	3-Br	41.57 ± 0.854	14.338 ± 1.098
6j	4-Br	35.89 ± 1.643	14.092 ± 0.313
6k	2-CH_3	38.09 ± 0.741	13.445 ± 2.188
6l	4-CH_3	33.21 ± 2.675	12.621 ± 0.235
6m	2-CN	83.57 ± 0.456	34.952 ± 2.032
6n	4-CN	73.55 ± 2.534	18.135 ± 0.134
6o	3-OCH_3	30.75 ± 1.965	9.118 ± 1.002
6p	4-OCH_3	33.17 ± 1.245	17.742 ± 0.424
6q	3-CF_3	36.79 ± 2.645	21.433 ± 1.955
6r	4-CF_3	35.28 ± 1.258	18.621 ± 1.456
6s	4-NO_2	66.20 ± 0.423	39.356 ± 0.644
Ascorbic acid	–	12.72 ± 0.274	5.0925 ± 0.2090

[a] The results are average of triplicate analysis.

3.2. Pharmacology

3.2.1. Antioxidant activities

DPPH· and $ABTS^{\cdot+}$ scavenging bioassay are the most common spectrophotometric methods to inspect the antioxidant power of the tested molecules. These free radicals interact with molecules subjected for testing which are capable to reduce the stable DPPH radical to the yellow colored diphenylpicrylhydrazine via donating a hydrogen due to the formation of the nonradical form, DPPH-H, during the reaction. To evaluate the free radical scavenging activity of **6a-s**, a DPPH assay was performed and the results are expressed in terms of IC_{50} value (concentration required to inhibit 50% of the radicals) as summarized in



R= H, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 3-Br, 2-CH_3, 4-CH_3, 2-CN, 4-CN, 3-OCH_3, 4-OCH_3, 3-CF_3, 4-CF_3, C-NO_2

Reagents and conditions: **(i)** H_2SO_4, MeOH; **(ii)** CH_3CN, reflux, 5 h; **(iii)** DMF, 3 h; **(iv)** $CuSO_{4.5}H_2O$, sodium ascorbate, DMF, 50 °C.

Scheme 1 Synthesis of substituted benzyl-1,2,3-triazolyl hesperetin derivatives **6a-s**.

Table 1. New flavanone derivatives **6a-s** possessed 30.75 ± 1.965 – 83.57 ± 0.456 μM and 9.118 ± 1.002 – 39.356 ± 0.644 μM of IC_{50} levels in DPPH and ABTS bioassay, respectively and can be comparable to ascorbic acid exerting 12.72 ± 0.274 μM (DPPH) and 5.0925 ± 0.2090 μM (ABTS) of IC_{50} levels. In general, titled scaffolds expressed encouraging results against $ABTS^{\cdot+}$ when compared to $DPPH^{\cdot}$. It was observed that types and position of electron withdrawing (EWD) or electron donating (ED) substituent played an essential role in delivering corresponding radical scavenging sensitivities. For example, molecules bearing EWD presented significant antioxidant power against DPPH radical, for example an analog **6o** carrying 3-OCH_3 group demonstrated 30.75 ± 1.965 μM of IC_{50} level. Moreover, **6l** bearing 4-CH_3 as well as **6p** holding 4-OCH_3 functionalities showed 33.21 ± 2.675 μM and 33.17 ± 1.245 μM of IC_{50} levels, respectively against DPPH. Overall data suggested that to achieve antioxidant activity against $DPPH^{\cdot}$ the nature of substituent was important than its position on the phenyl ring. However, in case of scaffolds carrying EWD halogen(s) groups, those with *para*-substitution were more active than their *meta*- and *ortho*-congeners. For example, **6j** with 4-Br group expressed 35.89 ± 1.643 μM of IC_{50} toward $DPPH^{\cdot}$, whereas **6h** (2-Br) and **6i** (3-Br) showed nearly 41 μM of IC_{50}. Compound **6a** with no substituent present on the phenyl ring displayed weak antioxidant power against $DPPH^{\cdot}$, and the fact was suggestive of the importance of placing appropriate substituent on the phenyl ring to achieve anticipated radical scavenging potencies. It was noticed that increasing number of halogen atoms resulted into the scaffolds exercising positively influenced antioxidant effects, as **6q** and **6r** with CF_3 group had 36.79 ± 2.645 μM and 35.28 ± 1.258 μM of IC_{50} levels, respectively and were higher than the antioxidant power of remaining halogen based analogs. Among EWD and ED based flavanones, antioxidant activity order can be presented as $OCH_3 > CH_3$ and $CF_3 > Br > F > Cl > NO_2 > CN$, respectively.

The $ABTS^{\cdot+}$ assay is based on a single electron transfer, the ABTS radical-cation decolorization, which is based on the reduction of $ABTS^{\cdot+}$ radicals by antioxidants. To evaluate the free radical scavenging activity of **6a-s**, ABTS assay was performed and the results are expressed in terms of IC_{50} value (concentration required to inhibit 50% of the radicals) as summarized in Table 1. Similar to DPPH assay, compound **6o** carrying 3-OCH_3 group had most potent scavenging activity against $ABTS^{\cdot+}$ with 9.118 ± 1.002 μM of IC_{50}, and was comparable to that of control ascorbic acid at 5.0925 ± 0.2090 μM. In fact, the position of the substituent had no influence of the corresponding $ABTS^{\cdot+}$ scavenging efficacies of **6a-s**. Analogs **6b** with 2-F and **6l** with 4-CH_3 expressed 12.645 ± 0.733 μM and 12.621 ± 0.235 μM of IC_{50} levels, respectively and were the second most active group of molecules against $ABTS^{\cdot+}$. Furthermore, it was found that analogs with EWD fluorine (**6d**) and ED methyl (**6k**) groups had contributed at a larger extent in presenting $ABTS^{\cdot+}$ sensitivities with IC_{50} levels nearby 13 μM. Bromine bearing scaffolds (**6i-6j**) demonstrated 13.445 ± 2.188 μM – 14.338 ± 1.098 μM of IC_{50} levels and can be comparable to the most active molecules in the series tested against $ABTS^{\cdot+}$. Opposite to DPPH assay, it was observed that increasing the number of halogen atoms had negative influence of the corresponding $ABTS^{\cdot+}$ scavenging efficacies of the result scaffolds as **6q** and **6r** had weak antioxidant effects with 21.433 ± 1.955 μM and 18.621 ± 1.456 μM of IC_{50} levels. Among EWD and ED based flavanone derivatives, the activity order can be placed as F > Br > Cl > CF_3 > -CN > NO_2 and $OCH_3 > CH_3$, respectively.

Table 2 Screening results of activity of **6a-s** against cervical cancer cell lines.

No.	R	IC_{50} μM ± SD[a]	IC_{50} μM ± SD[a]	CC_{50} μM ± SD[a]
		HeLa	CaSki	MDCK
6a	H	39.645 ± 1.234	52.822 ± 1.088	302.8 ± 2.348
6b	2-F	17.754 ± 0.754	27.644 ± 0.159	289.2 ± 1.573
6c	3-F	27.333 ± 0.674	22.535 ± 1.321	204.3 ± 1.907
6d	4-F	18.272 ± 0.544	15.453 ± 0.153	257.4 ± 0.394
6e	2-Cl	25.138 ± 1.556	33.274 ± 0.222	301.2 ± 2.571
6f	3-Cl	44.051 ± 0.986	31.546 ± 1.130	238.6 ± 3.422
6g	4-Cl	27.923 ± 0.578	42.832 ± 1.245	287.6 ± 2.575
6h	2-Br	33.456 ± 1.644	12.721 ± 0.256	198.9 ± 1.700
6i	3-Br	61.631 ± 1.463	15.547 ± 1.096	233.3 ± 0.207
6j	4-Br	38.574 ± 0.761	19.146 ± 0.134	261.4 ± 3.772
6k	2-CH_3	34.258 ± 2.003	16.046 ± 1.097	334.7 ± 2.792
6l	4-CH_3	37.731 ± 0.445	19.543 ± 0.146	329.1 ± 4.250
6m	2-CN	89.643 ± 0.613	67.634 ± 2.189	179.8 ± 1.415
6n	4-CN	69.354 ± 1.856	54.445 ± 1.097	237.1 ± 3.634
6o	3-OCH_3	56.753 ± 0.534	14.925 ± 0.078	290.9 ± 0.856
6p	4-OCH_3	61.083 ± 1.344	29.842 ± 2.269	312.2 ± 2.980
6q	3-CF_3	102.754 ± 1.521	27.329 ± 1.267	238.5 ± 5.931
6r	4-CF_3	74.521 ± 0.634	49.531 ± 0.129	273.3 ± 4.436
6s	4-NO_2	46.982 ± 0.744	77.841 ± 0.251	306.7 ± 1.162

[a] The results are average of triplicate analysis.

3.2.2. Anticancer activities

Analogs **6a-s** were screened in the three cancer cell lines panel consisting of HeLa and CaSki (cervical) as well as SK-OV-3 (ovarian). End point determinations were made with a protein binding dye, sulforhodamine B (SRB) called SRB assay and the results of anticancer screening tests for **6a-s** against cervical cancer cell lines are summarized in Table 2.

Overall results suggested that nature and position of the substituents had considerable impact of the anticancer effects of tested molecules. Compounds holding EWD halogen atoms were more active against HeLa cell line than their ED based congeners. For example, **6b** (2-F) and **6d** (4-F) expressed 17.754 ± 0.754 μM and 18.272 ± 0.544 μM of IC_{50} levels, respectively and were the most active anticancer agents against HeLa among all tested. These two molecules had tolerable cytotoxicity toward MDCK cell line with 289.2 ± 1.573 μM and 257.4 ± 0.394 μM of CC_{50} levels. The fact was suggestive of the importance of fluorine for potency against HeLa cell line, but it was noticed that increasing the number of fluorine atoms diminished the activity of corresponding scaffolds, for example **6q** and **6r** had 102.754 ± 1.521 μM and 74.521 ± 0.634 μM of IC_{50} levels against HeLa, respectively. Moreover, analogs **6e** and **6g** with chlorine group presented 25.138 ± 1.556 and 27.923 ± 0.578 μM of IC_{50} levels, and 301.2 ± 2.571 and 287.6 ± 2.575 μM of CC_{50} levels, respectively. The data suggested that *ortho*- and *para*-position has reasonable influence on the corresponding anticancer action of the resultant EWD based molecules against HeLa cell line. In addition, halogen based molecules holding meta-substitution were found poorly active against HeLa, as scaffolds **6c** (3-F), **6f** (3-Cl) and **6i** (3-Br) displayed 27.333

± 0.674 μM, 44.051 ± 0.986 μM and 61.631 ± 1.463 μM of IC_{50} levels, respectively. Among ED based flavanones, an analog with *ortho*-methyl substituent (**6k**) exerted 34.258 ± 2.003 μM of IC_{50}, but had significantly remarkable cytotoxicity toward MDCK cell line at 334.7 ± 2.792 μM of CC_{50}. In fact, its *para*-congener (**6l**) also showed 37.731 ± 0.445 μM of IC_{50} and can be considered to have desirable anticancer effects against HeLa cell line. Overall among EWD and ED based flavanones, anticancer effects against HeLa cell line can be respectively placed as F > Cl > Br > NO_2 > CF_3 > CN and CH_3 > OCH_3. The bioassay outcomes observed against CaSki cell lines were irrespective of the nature of the substituent present on the phenyl ring of these flavanones. For example, both EWD based **6d** (4-F) and ED based **6l** (4-CH_3) demonstrated 15.453 ± 0.153 and 19.543 ± 0.146 μM of IC_{50} levels, respectively. Likewise to bioassay results against HeLa cell line, increasing number of fluorine atom had negative effect on anticancer profiles of resultant analog, as CF_3 based **6q** and **6r** displayed 27.329 ± 1.267 μM and 49.531 ± 0.129 μM of IC_{50} levels, respectively. Furthermore, **6k** with 2-CH_3 functionality presented the best result as anticancer molecule among all tested in the series with 16.046 ± 1.097 μM of IC_{50}, whereas **6o** holding ED 3-OCH_3 group had 14.925 ± 0.078 μM of IC_{50}. These results suggested that ED group had achieved considerable attention in expressing anticancer effects against CaSki cell line than those carrying EWD groups unlike outcomes observed in SRB assay with HeLa cell line. Furthermore, bromine based flavanone (**6h**) appeared with lowest IC_{50} levels at 12.721 ± 0.256 μM, but was found to exercise somewhat cytotoxicity at 198.9 ± 1.700 μM. Another two bromine based molecules, **6i** and **6j** were succeeded to present 15.547 ± 1.096 μM and 19.146 ± 0.134 μM of IC_{50} levels, respectively, which were at remarkable level when compared to the potency of highly active molecules in the series. Overall among EWD and ED based flavanones, anticancer effects against CaSki cell line can be respectively placed as Br > F > Cl > CF_3 > CN > NO_2 and 2-CH_3 > 3-OCH_3 > 4-CH_3 > 4-OCH_3.

Analogs **6a-s** were tested to inspect their *in vitro* inhibitory efficacies against ovarian cancer cell line SK-OV-3 and the results of anticancer screening tests for **6a-s** are summarized in Table 3. Although, the activity profiles seen for **6a-s** against SK-OV-3 were weak when compared to those observed against cervical cancer cell lines, still the activity against SK-OV-3 was appreciable in terms of constructing detailed SAR analysis, as unlike above bioassay results, compounds **6q** bearing highly electronegative EWD 3-CF_3 group had 33.259 ± 1.534 μM of IC_{50}, 238.5 ± 5.931 μM of CC_{50}, which was very remarkable. These data implied that increasing number of halogen atoms may help to promote anticancer potential of the resultant molecules against ovarian cancer cell line. In addition, **6o** holding ED 3-OCH_3 expressed 41.479 ± 0.334 μM of IC_{50}, 290.9 ± 0.856 μM of CC_{50} and was also found to have high anticancer effects against SK-OV-3. It was also found that anticancer results of **6a-s** against SK-OV-3 had reversed than those observed against cervical cancer cell line in terms of position of substituents. For example, *meta*-position for EWD based molecules was found beneficial to exert anticancer effects against SK-OV-3, as **6c** (3-F), **6f** (3-Cl) and **6i** (3-Br) had 36.610 ± 1.270 μM, 36.792 ± 0.542 μM and 39.120 ± 1.239 μM of IC_{50} levels, respectively than that of their *ortho*- and *para*-substituted congeners at >43 μM. However, in case of ED methyl carrying molecules, compound holding *para*-substitution was observed to have better sensitivity in inhibiting SK-OV-3, as **6l** showed 56.438 ± 1.542 μM of IC_{50} when compared to that of **6k** (2-CH_3) at 59.875 ± 2.099 μM. Furthermore, unsubstituted phenyl ring was found to yield better results against SK-OV-3 than molecules holding NO_2 and CN functionalities, as **6a** had 61.651 ± 2.681 μM of IC_{50}, whereas **6m**, **6n** and **6s** displayed 78.541 ± 0.218 μM, 95.346 ± 0.911 μM and 65.115 ± 2.541 μM of IC_{50} levels, respectively. Overall among EWD and ED based flavanones, anticancer effects against SK-OV-3 cell line can be respectively placed as CF_3 > Cl > F > Br > NO_2 > CN and OCH_3 > CH_3.

Table 3 Screening results of activity of **6a-s** against ovarian cancer cell line.

No.	R	IC_{50} μM ± SD[a]	CC_{50} μM ± SD[a]
		SK-OV-3	MDCK
6a	H	61.651 ± 2.681	302.8 ± 2.348
6b	2-F	54.239 ± 1.923	289.2 ± 1.573
6c	3-F	36.610 ± 1.270	204.3 ± 1.907
6d	4-F	77.541 ± 1.456	257.4 ± 0.394
6e	2-Cl	47.229 ± 0.731	301.2 ± 2.571
6f	3-Cl	36.792 ± 0.542	238.6 ± 3.422
6g	4-Cl	56.873 ± 2.801	287.6 ± 2.575
6h	2-Br	43.665 ± 2.439	189.9 ± 1.700
6i	3-Br	39.120 ± 1.239	233.3 ± 0.207
6j	4-Br	64.542 ± 0.671	261.4 ± 3.772
6k	2-CH_3	59.875 ± 2.099	334.7 ± 2.792
6l	4-CH_3	56.438 ± 1.542	329.1 ± 4.250
6m	2-CN	78.541 ± 0.218	179.8 ± 1.415
6n	4-CN	95.346 ± 0.911	237.1 ± 3.634
6o	3-OCH_3	41.479 ± 0.334	290.9 ± 0.856
6p	4-OCH_3	47.580 ± 2.002	312.2 ± 2.980
6q	3-CF_3	33.259 ± 1.534	238.5 ± 5.931
6r	4-CF_3	49.561 ± 0.678	273.3 ± 4.436
6s	4-NO_2	65.115 ± 2.541	306.7 ± 1.162

[a] The results are average of triplicate analysis.

4. Conclusion

Copper-catalyzed azide–alkyne cycloaddition yielded 1,2,3-triazole core connecting a natural product flavanone hesperetin and substituted phenyls via click chemistry. With an aim to discover semi-synthetic flavanones with antioxidant and anticancer effects, titled scaffolds **6a-s** were screened for their DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ scavenging effects and cancerous cell inhibitory effects against cervical (HeLa and CaSki) and ovarian (SK-OV-3) cancer cell lines. From the bioassay outcomes it was noticed that nature and position of the EWD and ED substituents had remarkable effects. Molecules bearing ED groups demonstrated strong sensitivities in scavenging both DPPH and ABTS radicals and inhibiting CaSki cell line, whereas, those holding EWD groups presented excellent results against HeLa cell lines. Both EWD and ED based molecules exercised similar action against ovarian cancer cell line. In general, all the tested flavanone based 1,2,3-triazoles displayed good pharmacological potential and can be a tool to develop further set of highly potent antioxidant and anticancer agents.

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