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ORIGINAL ARTICLE

Evaluation of the biological potencies of newly synthesized berberine derivatives bearing benzothiazole moieties with substituted functionalities

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Abstract Benzothiazole moieties substituted with various functional groups were utilized to link with the isoquinoline alkaloid berberine through a pentyl side chain. Entitled analogs were screened for antioxidant potency using the DPPH and ABTS bioassays and for their *in vitro* anticancer activities against HeLa, CaSki (cervical cancer), and SK-OV-3 (ovarian cancer) cell lines using the SRB bioassay. The compounds were evaluated for their toxicity to the Madin–Darby canine kidney (MDCK) cell line. The final compounds demonstrated significant antioxidant potency with IC_{50} levels of 13.03–24.50 μg/mL and 4.958–7.570 μg/mL in the DPPH and ABTS radical scavenging bioassays, respectively. The **5e** analog with a methoxy functional group and the **5m** analog with a cyano functional group had the most significant DPPH and ABTS radical scavenging activities, respectively. Moreover, the **5m** cyano-based analog had the highest potency against all cancer cell lines, with IC_{50} levels of 5.474, 5.311, and 32.61 μg/mL against the HeLa, CaSki, and SK-OV-3 cell lines, respectively. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.

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1. Introduction

Diseases, such as diabetes, cirrhosis, cancer, and cardiovascular defects, are associated with the presence of free radicals [1,2]. Bioorganic redox reactions occurring in the human body introduce free radicals that produce oxidative damage to lipids, proteins, and nucleic acids [3]. Such oxidative damage can form mutations, causing several types of cancers. Hence, free radicals often harm the primary building blocks of human cells which create the seed for illnesses, such as cancers, hypertension, cardiac infarction, arteriosclerosis, and cataracts [4]. Homeostasis between free radicals and antioxidants is necessary for functioning, as oxidative stress arises if free radicals overcome the body's defenses.

Free radicals negatively modify lipids, proteins, and DNA and induce particular illnesses. Hence, applying an external source of antioxidants can help cope with this oxidative stress. Therefore, treatments for these pathophysiological conditions could benefit from the use of drugs that combine antioxidant and anti-inflammatory activities. Antioxidants are natural or synthetic molecules that defend against free radicals [5]. Thus, they can reverse illnesses, such as atherogenesis, carcinogenesis, inflammation, and aging [6,7]. Natural products have been an efficient source for the development of new medicines. Some new products are in clinical trials, particularly as anti-cancer and anti-infective agents. More than 80% of drugs were natural products or inspired by a natural compound [8]. Over 100 naturally derived substances are currently in clinical trials, and at least 100 are in preclinical development [9]. Most of these substances originated from plants and microbial sources [10]. Plant-based techniques play a significant role in healthcare [11]. The World Health Organization (WHO) estimated that about 65% of the world's population depended on plant-derived traditional medicines for primary medical care in 1985 [12].

Berberine is an isoquinoline alkaloid having a long history of therapeutic use in Ayurvedic and Chinese medicine. It occurs in the roots, rhizomes, and stem bark of *Hydrastis canadensis* (Goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). Berberine has significant activities against viruses, protozoans, fungi, and abdominal viruses. It is also helpful to treat microbial diarrhea, inflammation, thrombo-cytopenia, ventricular tachyarrhythmia, ocular trachoma infections, and sudden coronary death after ischemic myocardial damage [13]. Berberine also has important anticancer properties in human malignant brain tumors, esophageal cancer, and leukemic and colon cancers [14]. We attached a benzothiazole moiety to the berberine moiety to create semi-synthetic types of berberine. Benzothiazoles are heterocyclic compounds with various biological activities [15–21].

Considering the benefits of these heterocyclic nuclei, berberine and substituted benzothiazole moieties in a single molecular framework are candidate compounds to screen for biological activity. Thus, in this study, we report that this natural product skeleton can be used to develop novel agents with anticancer and antioxidant effects.

2. Experimental section

2.1. Materials and methods

The highest quality chemicals and reagents were used in this study without further purification. The VMP-D open capillary electronic apparatus (Veego Instruments) was utilized to obtain the uncorrected melting points of the synthesized compounds. A Shimadzu 8400-S Fourier-transform infrared (FT-IR) spectrophotometer (KBr pellets; Tokyo, Japan) and a Varian 500 MHz model spectrometer (Palo Alto, CA, USA) ($CDCl_3$ as the solvent and TMS as the internal standard) were used to obtain ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra of the title compounds. Thin-layer chromatography (TLC) was carried out using appropriate mobile phase systems and silica gel-G coated microscopic glass slides (2 × 7.5 cm), and the TLC spots were observed in a UV light chamber. FT-IR bands were presented in cm^{-1}, and the ^{1}H NMR spectral results were furnished in ppm downfield from TMS with s, singlet; d, doublet; m, multiplet and br s, broad singlet patterns. Elemental analyzes (C, H, and N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

2.2. General procedure for the synthesis of berberrubine (2)

Berberine chloride (10 g, 0.01 mol) was heated to 190 °C for 40 min under reduced pressure (20–30 mmHg) using an oil pump. The vacuum pump was turned off after the temperature dropped to room temperature to give a dark brown product. The product was purified by silica gel column chromatography ($CHCl_3$/CH_3OH:15:1 and 10:1, eluting until no compound was detected in the eluent) to obtain a brownish red amorphous powder compound 2 (8.5 g, 85%).

2.3. Synthesis of bromopentylberberrubine (3)

A solution of **2** (5 g, 0.01 mol) and 1,5-dibromopentan (0.01 mol) in dry acetonitrile was warmed at reflux temperature for 6 h and then diethyl ether was added. The resulting solid was filtered and then subject to anion-exchange into chloride form to give compound 3. Yield: 61%, m.p. 195–197 °C; IR (KBr) cm^{-1}: 3065, 1615, 1565, 1115–1050 (C–O–C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.86 (s, 1H, H-8), 8.49 (s, 1H, H-13), 7.86 (s, 1H, H-1), 7.54 (s, 1H, H-12), 7.54 (s, 1H, H-4), 6.84 (s, 1H, H-11), 6.03 (s, 2H, $-OCH_2O$), 4.93 (t, 2H, J = 6.3, H-6), 4.35 (t, 2H, J = 6.5, H-15), 4.03 (s, 3H, $-OCH_3$), 3.19 (t, 2H, J = 6.4, H-19), 2.58 (t, 2H, J = 7.4, H-5), 2.43 (br s, 4H, H-17, H-18), 2.21 (m, 2H, H-16); Anal. Calcd. for $C_{24}H_{25}BrClNO_4$: C, 56.88; H, 4.97; N, 2.76. Found: C, 56.75; H, 5.07; N, 2.54%.

2.4. General procedure for preparing the derivatives ***(5a–n)***

The substituent piperazine (0.01 mol) was added to a magnetically stirred solution of compound 3 and anhydrous K_2CO_3 in dry DMF (25 ml). The reaction mixture was heated at 80 °C for 6–8 h and monitored by TLC. The resulting solid was

filtered at room temperature and subjected to anion exchange into a chloride form. The crude product was chromatographed on an Al_2O_3 column and eluted with $CHCl_3/CH_3OH$ (9:1, v/v) to give the proposed compound.

*2.4.1. 9-O-3-(1-(2-Aminobenzthaizole)pentylberberine (**5a**)*

Light yellow solid, Yield: 65%. m.p. 256–258 °C. IR (KBr) cm^{-1}: 3408, 3028, 1641, 1529, 1448, 1102–1075; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.81 (s, 1H, H-8), 8.52 (s,1H, H-13), 8.31 (s, 1H, –NH), 7.96 (s, 1H, H-1), 7.77 (d, J = 1.9 Hz, 1H, benzothiazole ring), 7.60–7.51 (m, 3H, benzothiazole ring), 7.47 (s, 1H, H-12), 7.24 (s, 1H, H-4), 6.80 (s, 1H, H-11), 6.00 (s, 2H, $-OCH_2O$), 5.02 (t, 2H, J = 6.4, H-6), 4.36 (t, 2H, J = 6.6, H-15), 4.07 (s, 3H, OCH_3), 3.12 (t, 2H, J = 6.5, H-19), 2.54 (t, 2H, J = 7.5, H-5), 2.34 (br s, 4H, H-17, H-18), 2.09 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.3, 154.1, 152.2, 150.9, 147.4, 144.5, 142.0, 137.6, 135.7, 133.8, 129.3, 127.1, 126.0, 124.9, 123.2, 122.1, 119.2, 117.4, 115.5, 113.0, 109.1, 106.2, 103.3, 72.4, 60.5, 57.6, 54.7, 29.8, 28.9, 25.1, 23.2; Anal. Calcd. for $C_{31}H_{30}ClN_3O_4S$: C, 64.63; H, 5.25; N, 7.29. Found: C, 64.51; H, 5.17; N, 7.40.

*2.4.2. 9-O-3-(1-(6-Nitro-2-aminobenzthaizole)pentylberberine (**5b**)*

Light yellow solid, Yield: 67%. m.p. 261–263 °C. IR (KBr) cm^{-1}: 3417, 3025, 1624, 1578, 1512, 1109–1084; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.70 (s, 1H, H-8), 8.61 (s,1H, H-13), 8.42 (s, 1H, –NH), 7.86 (s, 1H, H-1), 7.82 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.67–7.58 (m, 2H, benzothiazole ring), 7.54 (s, 1H, H-12), 7.39 (s, 1H, H-4), 6.69 (s, 1H, H-11), 6.05 (s, 2H, $-OCH_2O$), 4.93 (t, 2H, J = 6.5, H-6), 4.25 (t, 2H, J = 6.4, H-15), 4.12 (s, 3H, OCH_3), 3.23 (t, 2H, J = 6.6, H-19), 2.59 (t, 2H, J = 7.4, H-5), 2.43 (br s, 4H, H-17, H-18), 2.14 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.8, 155.2, 153.1, 151.0, 148.3, 145.4, 143.5, 138.7, 136.9, 134.0, 130.8, 128.2, 126.2, 124.7, 122.5, 121.9, 120.1, 118.3, 116.4, 114.5, 110.0, 105.9, 104.2, 73.1, 61.6, 58.2, 55.8, 30.5, 28.7, 26.8, 23.4; Anal. Calcd. for $C_{31}H_{29}ClN_4O_6S$: C, 59.95; H, 4.71; N, 9.02. Found: C, 59.83; H, 4.84; N, 9.13.

*2.4.3. 9-O-3-(1-(2-Aminobenzothiazole-6-carboxylic acid) pentylberberine (**5c**)*

Light yellow solid, Yield: 59%. m.p. 249–251 °C. IR (KBr) cm^{-1}: 3461, 3074, 1747, 1652, 1534, 1453, 1112–1091; ^1H NMR ($CDCl_3$, 500 MHz): δ 11.39 (s, 1H, –OH), 9.76 (s, 1H, H-8), 8.57 (s,1H, H-13), 8.36 (s, 1H, –NH), 7.91 (s, 1H, H-1), 7.72 (d, J = 1.7 Hz, 1H, benzothiazole ring), 7.61–7.50 (m, 2H, benzothiazole ring), 7.41 (s, 1H, H-12), 7.29 (s, 1H, H-4), 6.75 (s, 1H, H-11), 5.95 (s, 2H, $-OCH_2O$), 4.98 (t, 2H, J = 6.3, H-6), 4.31 (t, 2H, J = 6.5, H-15), 4.03 (s, 3H, OCH_3), 3.18 (t, 2H, J = 6.4, H-19), 2.48 (t, 2H, J = 7.6, H-5), 2.39 (br s, 4H, H-17, H-18), 2.19 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 168.6, 161.5, 154.3, 152.4, 150.7, 147.6, 144.9, 142.2, 138.5, 136.3, 133.6, 129.5, 128.3, 126.9, 125.5, 123.4, 122.3, 119.4, 117.6, 116.9, 113.2, 109.3, 106.4, 103.5, 72.6, 60.7, 57.8, 54.9, 29.1, 27.2, 25.3, 24.1; Anal. Calcd. for $C_{32}H_{30}ClN_3O_6S$: C, 61.98; H, 4.88; N, 6.78. Found: C, 61.86; H, 4.96; N, 6.65.

*2.4.4. 9-O-3-(1-(6-Methyl-2-aminobenzthaizole) pentylberberine (**5d**)*

Light yellow solid, Yield: 57%. m.p. 247–249 °C. IR (KBr) cm^{-1}: 3368, 3066, 2939, 1634, 1547, 1461, 1105–1076; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.82 (s, 1H, H-8), 8.53 (s, 1H, H-13), 8.32 (s, 1H, –NH), 7.95 (s, 1H, H-1), 7.76 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.64–7.55 (m, 2H, benzothiazole ring), 7.52 (s, 1H, H-12), 7.25 (s, 1H, H-4), 6.81 (s, 1H, H-11), 6.01 (s, 2H, $-OCH_2O$), 5.03 (t, 2H, J = 6.3, H-6), 4.37 (t, 2H, J = 6.5, H-15), 4.08 (s, 3H, OCH_3), 3.13 (t, 2H, J = 6.4, H-19), 2.55 (t, 2H, J = 7.6, H-5), 2.35 (br s, 4H, H-17, H-18), 2.10 (m, 2H, H-16), 1.92 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.4, 155.4, 153.3, 151.2, 148.5, 145.6, 143.7, 137.8, 135.1, 134.1, 130.4, 127.4, 125.6, 124.1, 123.8, 121.2, 120.3, 118.5, 115.6, 114.7, 110.8, 105.7, 104.4, 73.3, 61.2, 58.4, 55.4, 30.7, 27.4, 26.6, 24.2, 20.8; Anal. Calcd. for $C_{32}H_{32}ClN_3O_4S$: C, 65.13; H, 5.47; N, 7.12. Found: C, 65.23; H, 5.56; N, 7.25.

*2.4.5. 9-O-3-(1-(6-Methoxy-2-aminobenzthaizole) pentylberberine (**5e**)*

Light yellow solid, Yield: 58%. m.p. 253–255 °C. IR (KBr) cm^{-1}: 3385, 3058, 2946, 1629, 1553, 1456, 1107–1088; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.71 (s, 1H, H-8), 8.62 (s,1H, H-13), 8.43 (s, 1H, –NH), 7.85 (s, 1H, H-1), 7.81 (d, J = 1.7 Hz, 1H, benzothiazole ring), 7.62–7.54 (m, 2H, benzothiazole ring), 7.46 (s, 1H, H-12), 7.38 (s, 1H, H-4), 6.70 (s, 1H, H-11), 6.06 (s, 2H, $-OCH_2O$), 4.94 (t, 2H, J = 6.4, H-6), 4.26 (t, 2H, J = 6.6, H-15), 4.13 (s, 3H, OCH_3), 3.92 (s, 3H), 3.24 (t, 2H, J = 6.5, H-19), 2.60 (t, 2H, J = 7.4, H-5), 2.44 (br s, 4H, H-17, H-18), 2.15 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.7, 154.5, 152.6, 150.5, 148.9, 144.7, 142.4, 137.4, 135.5, 133.4, 129.7, 127.5, 126.4, 125.3, 123.1, 121.7, 119.6, 118.9, 115.7, 113.4, 109.5, 106.6, 103.7, 73.5, 60.9, 57.1, 56.7, 54.3, 30.9, 28.5, 25.5, 23.6; Anal. Calcd. for $C_{32}H_{32}ClN_3O_5S$: C, 63.41; H, 5.32; N, 6.93. Found: C, 63.52; H, 5.40; N, 6.81.

*2.4.6. 9-O-3-(1-(6-Ethoxya-2-minobenzthaizole) pentylberberine (**5f**)*

Light yellow solid, Yield: 55%. m.p. 266–268 °C. IR (KBr) cm^{-1}: 3374, 3081, 2928, 1643, 1545, 1451, 1114–1093; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.77 (s, 1H, H-8), 8.58 (s, 1H, H-13), 8.37 (s, 1H, –NH), 7.90 (s, 1H, H-1), 7.71 (d, J = 1.9 Hz, 1H, benzothiazole ring), 7.66–7.52 (m, 2H, benzothiazole ring), 7.43 (s, 1H, H-12), 7.27 (s, 1H, H-4), 6.76 (s, 1H, H-11), 5.96 (s, 2H, $-OCH_2O$), 4.99 (t, 2H, J = 6.5, H-6), 4.32 (t, 2H, J = 6.4, H-15), 4.04 (s, 3H, OCH_3), 4.02 (m, 2H), 3.19 (t, 2H, J = 6.6, H-19), 2.49 (t, 2H, J = 7.5, H-5), 2.40 (br s, 4H, H-17, H-18), 2.20 (m, 2H, H-16), 1.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.2, 155.6, 153.5, 151.6, 147.8, 145.2, 143.9, 138.1, 136.7, 134.5, 130.2, 128.6, 126.7, 124.5, 122.6, 121.6, 120.5, 117.8, 116.2, 114.9, 110.6, 106.8, 104.6, 72.8, 67.5, 61.7, 57.3, 55.2, 29.3, 28.3, 26.4, 23.8, 19.2; Anal. Calcd. for $C_{33}H_{34}ClN_3O_5S$: C, 63.91; H, 5.53; N, 6.78. Found: C, 63.79; H, 5.44; N, 6.67.

*2.4.7. 9-O-3-(1-(6-Chloro-2-aminobenzthaizole)pentylberberine (**5g**)*

Light yellow solid, Yield: 60%. m.p. 269–271 °C. IR (KBr) cm^{-1}: 3451, 3045, 1656, 1539, 1449, 1106–1079, 769; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.83 (s, 1H, H-8), 8.54 (s, 1H, H-13), 8.33 (s, 1H, –NH), 7.94 (s, 1H, H-1), 7.75 (d, J = 1.9 Hz, 1H, benzothiazole ring), 7.63–7.54 (m, 2H, benzothiazole ring), 7.51 (s, 1H, H-12), 7.26 (s, 1H, H-4), 6.82 (s, 1H, H-11), 6.02 (s, 2H, $–OCH_2O$), 5.04 (t, 2H, J = 6.5, H-6), 4.38 (t, 2H, J = 6.4, H-15), 4.09 (s, 3H, OCH_3), 3.14 (t, 2H, J = 6.6, H-19), 2.56 (t, 2H, J = 7.5, H-5), 2.36 (br s, 4H, H-17, H-18), 2.11 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.6, 155.8, 153.7, 150.3, 147.1, 145.8, 143.1, 138.3, 136.1, 133.2, 129.6, 128.8, 126.5, 125.1, 123.3, 121.5, 119.7, 117.1, 116.8, 113.1, 110.4, 105.5, 103.9, 72.2, 60.3, 58.6, 54.5, 29.7, 27.6, 25.7, 24.3; Anal. Calcd. for $C_{31}H_{29}Cl_2N_3O_4S$: C, 60.98; H, 4.79; N, 6.88. Found: C, 61.04; H, 4.86; N, 6.79.

*2.4.8. 9-O-3-(1-(6-Bromo-2-aminobenzthaizole)pentylberberine (**5h**)*

Light yellow solid, Yield: 62%. m.p. 274–276 °C. IR (KBr) cm^{-1}: 3446, 3079, 1649, 1537, 1441, 1102–1082; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.72 (s, 1H, H-8), 8.63 (s, 1H, H-13), 8.44 (s, 1H, –NH), 7.89 (s, 1H, H-1), 7.80 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.65–7.53 (m, 2H, benzothiazole ring), 7.44 (s, 1H, H-12), 7.37 (s, 1H, H-4), 6.71 (s, 1H, H-11), 6.07 (s, 2H, $–OCH_2O$), 4.95 (t, 2H, J = 6.3, H-6), 4.27 (t, 2H, J = 6.5, H-15), 4.14 (s, 3H, OCH_3), 3.25 (t, 2H, J = 6.4, H-19), 2.61 (t, 2H, J = 7.6, H-5), 2.45 (br s, 4H, H-17, H-18), 2.16 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.9, 154.7, 152.8, 151.8, 148.7, 144.3, 142.6, 137.2, 135.9, 133.9, 130.9, 127.7, 125.7, 124.2, 122.8, 121.6, 120.8, 118.7, 115.3, 114.6, 109.7, 105.2, 104.1, 73.7, 61.9, 58.8, 55.7, 30.8, 27.8, 26.2, 24.5; Anal. Calcd. for $C_{31}H_{29}BrClN_3O_4S$: C, 56.84; H, 4.46; N, 6.42. Found: C, 56.72; H, 4.38; N, 6.51.

*2.4.9. 9-O-3-(1-(6-Iodo-2-aminobenzthaizole)pentylberberine (**5i**)*

Light yellow solid, Yield: 56%. m.p. 252–254 °C. IR (KBr) cm^{-1}: 3459, 3062, 1639, 1542, 1445, 1115–1094; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.78 (s, 1H, H-8), 8.59 (s, 1H, H-13), 8.38 (s, 1H, –NH), 7.89 (s, 1H, H-1), 7.70 (d, J = 1.7 Hz, 1H, benzothiazole ring), 7.60–7.52 (m, 2H, benzothiazole ring), 7.49 (s, 1H, H-12), 7.23 (s, 1H, H-4), 6.77 (s, 1H, H-11), 5.97 (s, 2H, $–OCH_2O$), 5.00 (t, 2H, J = 6.4, H-6), 4.33 (t, 2H, J = 6.6, H-15), 4.05 (s, 3H, OCH_3), 3.20 (t, 2H, J = 6.5, H-19), 2.50 (t, 2H, J = 7.4, H-5), 2.41 (br s, 4H, H-17, H-18), 2.21 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.2, 154.9, 152.1, 150.1, 148.2, 144.1, 142.8, 137.9, 135.3, 133.1, 130.7, 128.9, 126.3, 124.8, 123.6, 122.4, 120.1, 118.2, 116.1, 113.8, 109.9, 106.1, 103.2, 73.6, 60.1, 57.5, 54.1, 30.6, 28.1, 25.9, 23.1; Anal. Calcd. for $C_{31}H_{29}ClIN_3O_4S$: C, 53.04; H, 4.16; N, 5.99. Found: C, 53.15; H, 4.25; N, 6.07.

*2.4.10. 9-O-3-(1-(6-Fluoro-2-aminobenzthaizole) pentylberberine (**5j**)*

Light yellow solid, Yield: 55%. m.p. 260–262 °C. IR (KBr) cm^{-1}: 3465, 3067, 1630, 1531, 1450, 1108–1095; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.89 (s, 1H, H-8), 8.55 (s, 1H, H-13), 8.34 (s, 1H, –NH), 7.93 (s, 1H, H-1), 7.74 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.67–7.59 (m, 2H, benzothiazole ring), 7.53 (s, 1H, H-12), 7.28 (s, 1H, H-4), 6.83 (s, 1H, H-11), 6.03 (s, 2H, $–OCH_2O$), 5.05 (t, 2H, J = 6.4, H-6), 4.39 (t, 2H, J = 6.6, H-15), 4.10 (s, 3H, OCH_3), 3.15 (t, 2H, J = 6.5, H-19), 2.57 (t, 2H, J = 7.4, H-5), 2.37 (br s, 4H, H-17, H-18), 2.12 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.1, 155.1, 153.9, 151.7, 147.3, 145.9, 142.5, 138.2, 136.5, 134.7, 129.1, 127.2, 125.9, 125.2, 123.5, 121.1, 119.9, 117.3, 115.9, 114.5, 110.1, 106.3, 104.3, 72.9, 61.5, 57.7, 55.3, 29.9, 27.1, 26.7, 24.7; Anal. Calcd. for $C_{31}H_{29}ClFN_3O_4S$: C, 62.67; H, 4.92; N, 7.07. Found: C, 62.56; H, 4.84; N, 7.16.

*2.4.11. 9-O-3-(1-(4, 6-Difluoro-2-aminobenzthaizole) pentylberberine (**5k**)*

Light yellow solid, Yield: 58%. m.p. 284–286 °C. IR (KBr) cm^{-1}: 3435, 3085, 1637, 1528, 1463, 1111–1085; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.73 (s, 1H, H-8), 8.64 (s, 1H, H-13), 8.45 (s, 1H, –NH), 7.98 (s, 1H, H-1), 7.79 (d, J = 1.9 Hz, 1H, benzothiazole ring), 7.65 (s, 1H, benzothiazole ring), 7.42 (s, 1H, H-12), 7.38 (s, 1H, H-4), 6.72 (s, 1H, H-11), 6.08 (s, 2H, $–OCH_2O$), 4.96 (t, 2H, J = 6.3, H-6), 4.28 (t, 2H, J = 6.5, H-15), 4.15 (s, 3H, OCH_3), 3.26 (t, 2H, J = 6.6, H-19), 2.62 (t, 2H, J = 7.5, H-5), 2.46 (br s, 4H, H-17, H-18), 2.17 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.4, 155.3, 153.2, 150.8, 148.4, 145.5, 143.2, 137.7, 135.0, 133.3, 129.4, 128.3, 126.6, 125.4, 123.9, 122.2, 119.2, 118.4, 116.5, 113.2, 110.3, 105.4, 103.8, 73.2, 60.8, 58.1, 54.8, 30.4, 28.0, 25.8, 23.5; Anal. Calcd. for $C_{31}H_{28}ClF_2N_3O_4S$: C, 60.83; H, 4.61; N, 6.87. Found: C, 60.94; H, 4.54; N, 6.96.

*2.4.12. 9-O-3-(1-(6-Trifluoromethyl-2-aminobenzthaizole) pentylberberine (**5l**)*

Light yellow solid, Yield: 57%. m.p. 273–275 °C. IR (KBr) cm^{-1}: 3470, 1625, 1583, 1478, 1427, 1174, 1129, 1106–1096 (C–O–C); ^1H NMR ($CDCl_3$, 500 MHz): δ 9.79 (s, 1H, H-8), 8.60 (s, 1H, H-13), 8.39 (s, 1H, –NH), 7.88 (s, 1H, H-1), 7.69 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.64–7.56 (m, 2H, benzothiazole ring), 7.50 (s, 1H, H-12), 7.30 (s, 1H, H-4), 6.78 (s, 1H, H-11), 5.98 (s, 2H, $–OCH_2O$), 5.01 (t, 2H, J = 6.5, H-6), 4.34 (t, 2H, J = 6.4, H-15), 4.06 (s, 3H, OCH_3), 3.21 (t, 2H, J = 6.4, H-19), 2.51 (t, 2H, J = 7.6, H-5), 2.42 (br s, 4H, H-17, H-18), 2.22 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.3, 154.6, 152.5, 151.5, 147.5, 144.8, 142.1, 138.4, 135.8, 134.2, 130.3, 127.6, 126.1, 125.3, 124.6, 122.7, 121.8, 120.5, 117.5, 115.8, 114.1, 109.0, 106.5, 104.5, 72.7, 61.3, 58.5, 55.1, 29.5, 28.8, 26.5, 24.9; Anal. Calcd. for $C_{32}H_{29}ClF_3N_3O_4S$: C, 59.67; H, 4.54; N, 6.52. Found: C, 59.55; H, 4.49; N, 6.43.

*2.4.13. 9-O-3-(1-(6-Cyno-2-aminobenzthaizole)pentylberberine (**5m**)*

Light yellow solid, Yield: 54%. m.p. 280–282 °C. IR (KBr) cm^{-1}: 3475, 2278, 1633, 1588, 1485, 1436, 1163, 1132, 1104–1089 (C–O–C); ^1H NMR ($CDCl_3$, 500 MHz): δ 9.85 (s, 1H, H-8), 8.56 (s, 1H, H-13), 8.35 (s, 1H, –NH), 7.92 (s, 1H, H-1), 7.73 (d, J = 1.8 Hz, 1H, benzothiazole ring), 7.62–7.55 (m, 2H, benzothiazole ring), 7.47 (s, 1H, H-12), 7.37 (s, 1H, H-4), 6.84 (s, 1H, H-11), 6.04 (s, 2H, $–OCH_2O$), 5.06 (t, 2H, J = 6.4, H-6), 4.40 (t, 2H, J = 6.6, H-15), 4.11 (s, 3H,

OCH_3), 3.16 (t, 2H, J = 6.5, H-19), 2.58 (t, 2H, J = 7.5, H-5), 2.38 (br s, 4H, H-17, H-18), 2.13 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 161.6, 155.5, 152.7, 150.4, 148.8, 145.3, 143.8, 137.5, 136.6, 133.5, 130.6, 128.5, 126.8, 124.4, 123.0, 122.4, 120.5, 119.7, 117.8, 116.3, 113.8, 110.5, 105.6, 103.4, 73.8, 60.2, 57.9, 54.0, 30.2, 27.7, 25.6, 23.7; Anal. Calcd. for $C_{32}H_{29}ClN_4O_4S$: C, 63.93; H, 4.86; N, 9.32. Found: C, 63.85; H, 4.78; N, 9.46.

2.4.14. 9-O-3-(1-(2-Hydrazinobenzthaizole)pentylberberine (**5n**)

Light yellow solid, Yield: 58%. m.p. 271–273 °C. IR (KBr) cm^{-1}: 3468, 1631, 1591, 1473, 1429, 1171, 1114–1081 (C–O–C); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.74 (s, 1H, H-8), 8.65 (s, 1H, H-13), 8.46 (s, 1H, –NH), 8.29 (s, 1H, –NH), 7.97 (s, 1H, H-1), 7.78 (d, J = 1.7 Hz, 1H, benzothiazole ring), 7.66–7.53 (m, 3H, benzothiazole ring), 7.44 (s, 1H, H-12), 7.31 (s, 1H, H-4), 6.73 (s, 1H, H-11), 6.09 (s, 2H, $-OCH_2O$), 4.97 (t, 2H, J = 6.3, H-6), 4.29 (t, 2H, J = 6.5, H-15), 4.16 (s, 3H, OCH_3), 3.27 (t, 2H, J = 6.6, H-19), 2.63 (t, 2H, J = 7.4, H-5), 2.47 (br s, 4H, H-17, H-18), 2.18 (m, 2H, H-16); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 162.5, 154.8, 153.4, 151.1, 147.7, 144.6, 143.4, 138.6, 135.2, 134.6, 129.5, 127.8, 125.8, 125.0, 122.9, 121.3, 120.4, 117.7, 115.6, 114.4, 109.2, 106.7, 104.9, 72.3, 61.1, 58.7, 55.8, 29.6, 28.2, 26.3, 24.1; Anal. Calcd. for $C_{31}H_{31}ClN_4O_4S$: C, 62.99; H, 5.29; N, 9.48. Found: C, 62.88; H, 5.38; N, 9.60.

2.5. DPPH free radical scavenging assay

Reduction of the stable 2,2-diphenyl-1-picrylhydrazyl free radical is the basis of the DPPH antioxidant bioassay. This compound has an odd electron that exerts a maximum absorption band of 517 nm (deep violet color) in ethanol. Substances donate a hydrogen atom when mixed with DPPH thereby introducing the reduced diphenyl picrylhydrazine (non-radical) congener and loss of the violet color. The results of the DPPH bioassay are presented as a percentage of radical scavenging antioxidant activity (RSA%) of each substance. DPPH radical scavenging activity of the berberine derivatives **5a–n** were determined according to a method described previously [22,23]. The berberine derivatives (20 μL) were added to a 96-well microplate with 180 μL of DPPH. Methanol (20 μL) was used as the blank and optical density was determined at 517 nm after a 30 min incubation. The control contained all reagents except the scavenger. The DPPH radical scavenging activity of ascorbic acid was assayed for comparison, and all tests were performed in triplicate. The RSA% results were determined according to Mensor et al. [26] as described in the equation:

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

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

2.6. ABTS radical scavenging assay

The $ABTS^{\cdot+}$ radical cation scavenging efficacies of the test compounds were determined according to a method described earlier [23,24]. In brief, 20 μL of sample was combined with 180 μL of ABTS radical solution followed by a 10 min incubation in the dark, and absorbance was measured at 734 nm. Ascorbic acid was used as a reference. The UV absorption data represented the radical scavenging rates and the corresponding IC_{50s} for the test compounds.

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

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

2.7. In vitro anticancer bioassay

2.7.1. Cell cultures

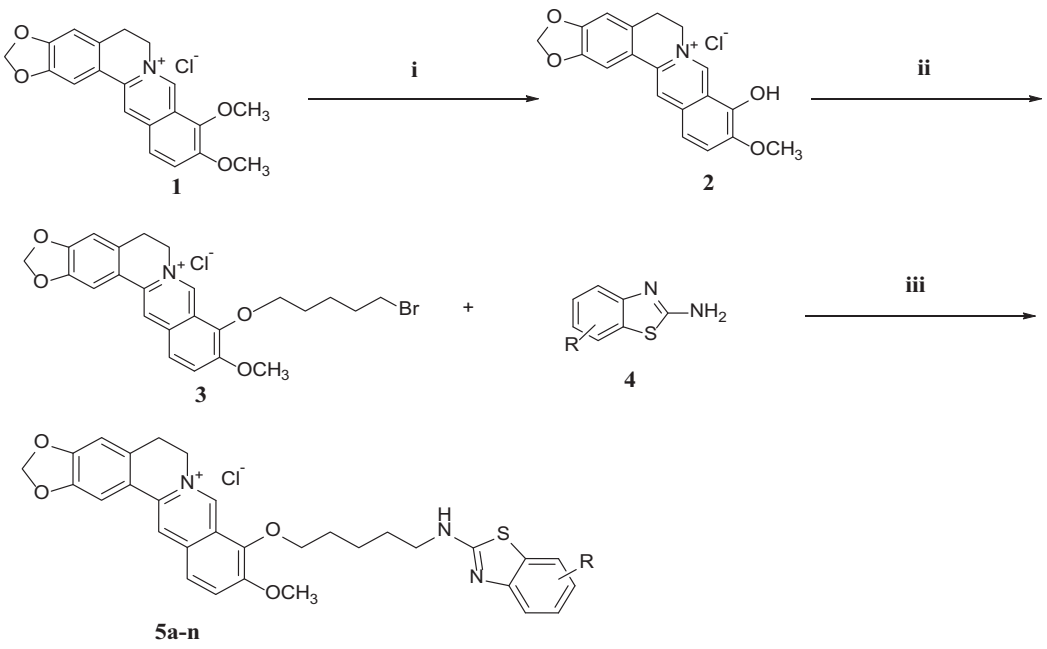
Test compounds **5a–n** were checked for their *in vitro* anticancer activities against the HeLa and CaSki cervical cancer cell lines, SK-OV-3 ovarian cancer cell lines and Madin–Darby canine kidney (MDCK) cells (American Type Culture Collection, Manassas, VA, USA). All cells were maintained in a humidified incubator in 5% CO_2 at 32 °C. Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium supplemented with 10% of fetal bovine serum (FBS) and 1% Antibiotic–Antimycotic Solution (100X) were used. DMEM, RPMI-1640, trypsin-EDTA, Antibiotic–Antimycotic Solution, and FBS were purchased from Welgene (Daegu, Republic of Korea).

The cells (2×10^4 cells/well) were seeded in 96-well plates and allowed to grow for 1 day. Then, the 96-well plates were washed twice with phosphate buffer saline (PBS). Aliquots of 0.1, 1, 10, and 100 μL of the test compounds were added to the plates in triplicate and incubated for 48 h. After incubation, the medium was removed, and the cells were washed twice with PBS. Then, 70% acetone was added to fix the cells and incubated for 1 h at 4 °C. After the incubation, the solvent was removed, and the plates were dried in an oven at 60 °C. The dried plates were incubated overnight in 100 μL SRB (0.4 mg/L). Then, the SRB was removed, the plates were washed three times with 1% acetic acid, and dried again at 60 °C. Microscopic observations were carried out to determine cell morphology. The SRB was dissolved with 10 mM Tris base and incubated overnight. [23,25] Spectrophotometric data were recorded at 510 nm to calculate the IC_{50}, and the 50% cytotoxic concentration (CC_{50}).

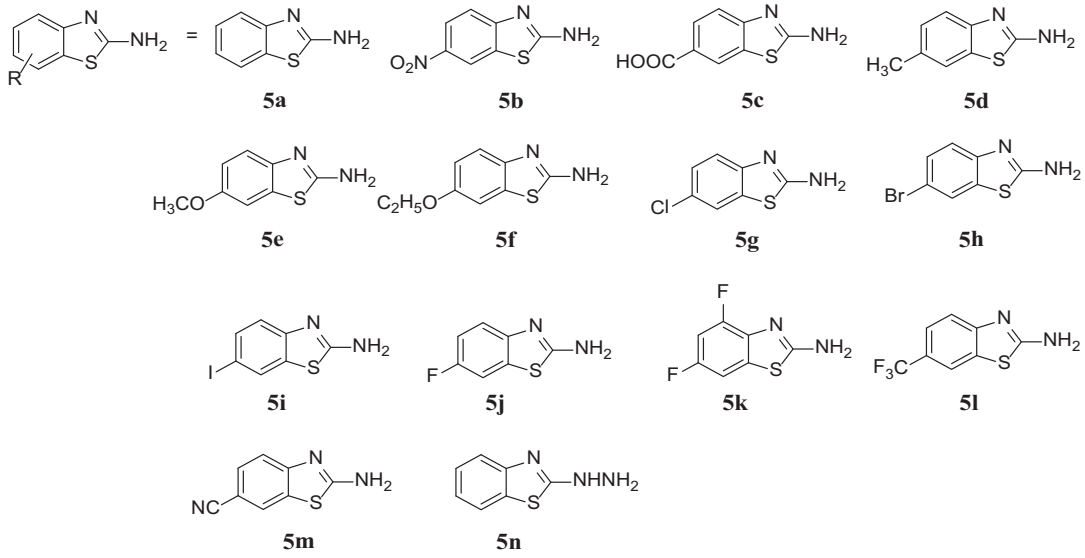
3. Results and discussion

3.1. Chemistry

Scheme 1 reveals the synthesis of the final berberine–benzothiazole derivatives (**5a–n**). Demethylation of berberine chloride under vacuum at 190 °C and 20–30 mm Hg pressure provided an 85% berberrubine (**2**) yield [26]. Treatment of 1,5-dibromopentane [27] with berberrubine (**2**) produced the final intermediate derivative **3** to carry out a nucleophilic replacement with a variety of heterocyclic compounds that



Reagents & conditions: i. 190°C, 20-30 mm Hg, 40 min: ii. CH_3CN, 1,5-dibromopentan, reflux, 6h: iii. K_2CO_3, DMF, reflux, 6-8 h.



Scheme 1 Synthesis of benzothiazole linked berberine derivatives.

replaced the benzothiazole groups (**4**) to produce the **5a–n** molecules and 2-amino-6-substituted benzothiazoles (**4**) were yielded in reasonably good yields by treating corresponding amines with KSCN in glacial acetic acid in the presence of bromine adopting known preparation method [15,28,29].

Synthesis of the proper structures of compounds **5a–n** was verified using FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The FT-IR absorption bands of **5d** at 3066 cm^{-1} and 2939 cm^{-1} verified the existence of C–H and C–C stretching for aromatics, respectively, whereas the C=C band of aromatics provided groups at 1547 cm^{-1} and 1461 cm^{-1} along with the appearance of the NH proton at 3368 cm^{-1}. The C–O–C band exhibited an intense absorption band at 1105–1076 cm^{-1} and bands occurring at 1634 cm^{-1} were allocated to the –C=N stretching vibrations of the benzothiazole molecule. Further evidence was obtained from the ^{1}H NMR spectrum of compound (**5d**) proton atoms, which revealed the presence of expected signals corresponding to berberine ring singlets at 9.82, 8.53, 7.95, 7.52, 7.25 and 6.81 ppm for the H-8, H-13, H-1, H-12, H-4, and H-11 protons, respectively. The presence of signal singlet peaks at 8.32 ppm was attributed to NH functionalities, and a singlet appeared at 6.01 ppm for the $–OCH_2O–$ protons in the berberine ring. Significant peaks were observed as a triplet at 5.03 ppm and 2.55 ppm for H-6

and H-5 of the berberine ring. An alkyl chain proton dropped at around 4.37 ppm by means of a triplet. In addition, the triplet signal at 3.13 ppm, the broad singlet at 2.35 ppm, and the multiplet signals at 2.10 ppm were due to the alkyl chain protons H-19, H-17, H-18, and H-16, respectively. The proton atoms from the methoxy functional group resonated at 4.08 ppm. Additionally, a doublet at 7.76 ppm was allocated to the benzothiazole ring and multiplets were detected at 7.64–7.55 ppm from the remaining two protons of the benzothiazole ring. The ^{13}C NMR spectra provided further details than those acquired from the IR and ^{1}H NMR spectra. All novel compounds produced C, H, and N values within 0.4% from the theoretical values, which was acceptable.

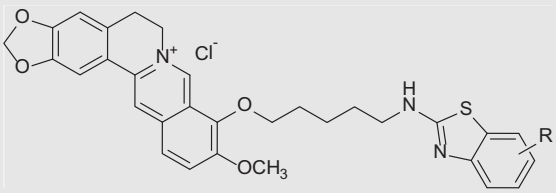
3.2. Evaluation of biological activities

3.2.1. Antioxidant activities

The antioxidant activities of the synthesized benzothiazole-based berberine scaffolds were evaluated using DPPH and ABTS radical scavenging bioassays and the results are summarized in Table 1. The antioxidant activities of the benzothiazole-based berberine scaffolds varied considerably depending on the backbone structure and functional groups attached to the berberine via the pentyl side chain. Overall, derivatized berberine exerted increased potency in both antioxidant bioassays compared to that of the analog parent berberine itself. Different electron withdrawing functional groups, such as chloro, fluoro, nitro, and trifluoromethyl, as well as electron donating functional groups, such as methyl and methoxy, were present on the piperazine moieties attached to the berberine ring. Overall, highly significant antioxidant efficacies were observed for compounds **5a–n** compared to those of the parent molecule berberine. The **5a–n** scaffolds had IC_{50} values of 13.03 ± 0.086–24.50 ± 1.903 μg/mL and 4.958 ± 0.065–7.570 ± 0.189 μg/mL in the DPPH and ABTS bioassays, respectively compared to those of the berberine (38.00 ± 1.722 and 82.90 ± 1.170 μg/mL) and were comparable to that of ascorbic acid control (12.22 ± 0.106 and 5.042 ± 0.045 μg/mL, respectively). The efficacies of **5a–n** as antioxidant agents were better in the ABTS than those in the DPPH assay. These results suggest that varying the substituents attached to the pentyl side chain on the berberine core played a significant role in the overall radical scavenging potencies of the molecules.

Scaffold **5e** bearing the electron-donating methoxy functional group was the most potent, with an IC_{50} to scavenge DPPH radicals of 13.03 ± 0.086 μg/mL, compared to ascorbic acid (12.22 ± 0.106 μg/mL). Furthermore, the presence of electron withdrawing halogen atoms, such as chlorine (**5g**) and bromine (**5h**), on the C-6 position of the 2-aminobenzothazole ring attached to the berberine core was effective for scavenging DPPH radicals, with IC_{50} values of 15.58 ± 0.635 and 16.88 ± 1.233 μg/mL, respectively. In addition, the analog with the EWD cyano group (**5m**) exerted noticeable DPPH scavenging activity, with an IC_{50} of 14.95 ± 0.355 μg/mL, which was the second most potent derivative against DPPH tested. The presence of two fluorine atoms increased DPPH scavenging effects of **5k** compared to that of its mono-substituted scaffold (**5j**), with IC_{50} values of 16.40 ± 0.007 and 18.43 ± 0.534 μg/mL, respectively. Analogs bearing acidic (**5c**) and trifluoromethyl (**5l**) groups presented IC_{50} values of nearly 17 μg/mL. In general, all the final compounds exhibited significantly higher DPPH radical scavenging activity compared to the IC_{50} value observed for the parent berberine molecule (12.22 ± 0.106 μg/mL) in the DPPH bioassay. Moreover, the compound with the EWD cyano group (**5m**) yielded an IC_{50} of 4.958 ± 0.065 μg/mL in the ABTS assay which was higher than that of ascorbic acid (5.042 ± 0.045 μg/mL). Several other derivatives, such as **5c–5g**, with either EWD or ED groups had IC_{50} values of 5 μg/mL, which was comparable to ascorbic acid and much better than berberine against ABTS radicals. In fact, scaffolds bearing ED groups were more active in both antioxidant assays than compounds bearing halogen atom(s), except an analog carrying a cyano group. Overall, all compounds demonstrated increased scavenging effects against the DPPH and ABTS radicals compared to that of berberine, whereas other compounds exhibited similar DPPH and ABTS radical scavenging activities like that of the ascorbic acid control.

Table 1 Screening results of DPPH and ABTS radical scavenging activity of berberine derivatives **(5a–n)**.



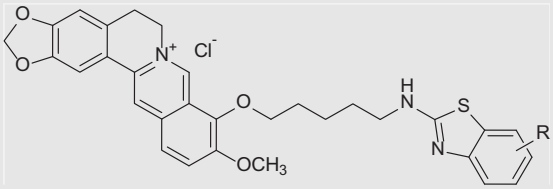
No.	R	[a]IC_{50} μg/ml ± SD	
		[b]DPPH	[c]ABTS
5a	H	24.50 ± 1.903	6.755 ± 0.088
5b	6-NO_2	23.29 ± 1.436	7.570 ± 0.189
5c	6-COOH	17.12 ± 1.290	5.363 ± 0.101
5d	6-CH_3	16.21 ± 1.033	5.403 ± 0.027
5e	6-OCH_3	13.03 ± 0.086	5.064 ± 0.072
5f	6-OC_2H_5	19.48 ± 1.336	5.504 ± 0.129
5g	6-Cl	15.58 ± 0.635	5.629 ± 0.096
5h	6-Br	16.88 ± 1.233	6.042 ± 0.159
5i	6-I	22.34 ± 1.267	6.406 ± 0.243
5j	6-F	18.43 ± 0.534	6.134 ± 0.075
5k	4,6-di F	16.40 ± 0.007	5.947 ± 0.023
5l	6-CF_3	17.91 ± 1.019	6.031 ± 0.012
5m	6-CN	14.95 ± 0.355	4.958 ± 0.065
5n	–	20.83 ± 0.836	5.625 ± 0.093
Berberine		38.00 ± 1.722	82.90 ± 1.170
Ascorbic acid		12.22 ± 0.106	5.042 ± 0.045

[a] Antioxidant activities are shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results expressed as an average ± standard deviation.
[b] DPPH = 2,2-diphenyl-1-picrylhydrazyl.
[c] ABTS = 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid.

3.2.2. Anticancer activities

Analogs **5a–n** were examined for their *in vitro* anticancer potencies in HeLa (cervical), CaSki (cervical), and SK-OV-3 (ovarian) cells using the SRB bioassay as shown in

Table 2 Screening results of activity of **5a–n** against cervical cancer cell lines.



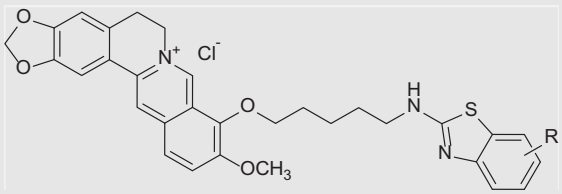
No.	R	[a]IC_{50} μg/ml ± SD[a]	[a]IC_{50} μg/ml ± SD	[b]CC_{50} μg/ml ± SD
		HeLa	CaSki	MDCK
5a	H	6.409 ± 0.059	6.807 ± 0.277	306.4 ± 0.886
5b	6-NO_2	6.082 ± 0.014	6.377 ± 0.440	303.6 ± 0.059
5c	6-COOH	6.198 ± 0.104	6.584 ± 0.306	308.1 ± 0.410
5d	6-CH_3	5.985 ± 0.060	6.459 ± 0.069	309.1 ± 0.233
5e	6-OCH_3	5.878 ± 0.043	5.974 ± 0.083	339.0 ± 0.412
5g	6-Cl	5.844 ± 0.026	5.876 ± 0.359	333.9 ± 0.624
5h	6-Br	6.087 ± 0.114	6.488 ± 0.262	318.4 ± 1.452
5i	6-I	6.324 ± 0.073	6.724 ± 0.421	304.5 ± 0.924
5j	6-F	6.264 ± 0.265	6.653 ± 0.282	308. 6 ± 0.628
5k	4,6-di F	5.913 ± 0.064	5.661 ± 0.352	314.2 ± 0.663
5l	6-CF_3	6.465 ± 0.011	6.771 ± 0.300	321.2 ± 3.275
5m	6-CN	5.474 ± 0.019	5.311 ± 0.164	312.8 ± 1.206
5n	–	6.406 ± 0.039	6.882 ± 0.255	307.0 ± 1.425
Berberine		5.700 ± 0.026	5.676 ± 0.137	111.8 ± 0.658

[a] Anticancer activities are shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results expressed as an average ± standard deviation.
[b] CC_{50} – cytotoxicity concentration of 50%.

Tables 2 and 3. All compounds exhibited reasonable anticancer potencies against HeLa, CaSki, and SK-OV-3 cells based on the activity level observed for parent compound berberine. In fact, benzothiazole substitution via the pentyl chain revealed similar anticancer activities. The IC_{50} values were 5.474 ± 0.019–6.465 ± 0.011 μg/mL and 5.311 ± 0.164–6.882 ± 0.255 μg/mL against HeLa and CaSki cells, respectively compared to that of berberine itself, which had IC_{50} values of 5.700 ± 0.026 (HeLa) and 5.676 ± 0.137 μg/mL (CaSki). Varying the functional groups attached to the benzothiazole ring led to the changes in anticancer efficacies of the resulting molecules. Secondly, final compounds **5a-n** exerted tolerable cytotoxic nature with cytotoxicity values ranging from 303.6 ± 0.059 to 339.0 ± 0.412 μg/mL against MDCK cell lines.

Similarly, the **5e** analog with an electron-donating methoxy functional group had an IC_{50} value of 5.878 ± 0.043, a CC_{50} value of 339.0 ± 0.412 μg/mL against HeLa cells and was the most potent molecule compared to that of berberine (IC_{50} = 5.700 ± 0.026 μg/mL, CC_{50} = 111.8 ± 0.658 μg/mL). Moreover, the same compound had an IC_{50} value of 5.974 ± 0.083 μg/mL against CaSki cells. In contrast, analogs bearing an EWD halogen atom in the form of chlorine (**5g**) had IC_{50} values of 5.844 ± 0.026 and 5.876 ± 0.359 μg/mL, respectively against HeLa and CaSki cells. This chlorine based derivative carried very tolerable cytotoxic nature with 333.9 ± 0.624 μg/mL of CC_{50} toward non-cancer MDCK cell line. The scaffold with a cyano functional group (**5m**) also had anticancer activity, with IC_{50} values of 5.474 ± 0.019 and 5.311 ± 0.164 μg/mL against HeLa and CaSki cells, respectively, a CC_{50} value of 312.8 ± 1.206 μg/mL against HeLa and CaSki cells. This was, in fact, the most potent compound against CaSki cell lines among all tested in the series when compared to the IC_{50} of berberine against CaSki at 5.676 ± 0.137 μg/mL. Moreover, analogs with ED methyl group (**5d**), EWD bromine atom (**5h**) as well as with two EWD fluorine atoms (**5k**) demonstrated 5.985 ± 0.060 μg/mL, 6.087 ± 0.114 μg/mL and 5.913 ± 0.064 μg/mL of IC_{50} levels, respectively against HeLa cell line. Interestingly, analogs (**5k**) exhibited 5.661 ± 0.352 μg/mL of IC_{50} against CaSki cell line. These analogs are observed to have good to noticeable effects against both the cervical cancer cell lines. All the remaining analogs were still noticed to manage higher anticancer effects in terms of IC_{50s} observed against HeLa and CaSki at nearby 6 μg/mL when compared to that of berberine at around 5.7 μg/mL. Final compounds **5a-n** were observed to yield moderate level of anticancer effects against ovarian cancer cell line SK-OV-3. Newly furnished scaffolds exhibited 32.61 ± 0.581–55.94 ± 0.890 μg/mL of IC_{50}s against SK-OV-3 cell line. Similar to results obtained from cervical cancer cell lines, a compound with EWD cyano functional group (**5m**) exhibited the highest level of anticancer effects against SK-OV-3 with 32.61 ± 0.581 μg/mL of IC_{50} and 312.8 ± 1.206 μg/mL of CC_{50}s, which was comparable to that of berberine at 24.17 ± 1.196 μg/mL of IC_{50}. Moreover, an analog with ED methoxy group (**5e**) was found to exert 37.52 ± 0.469 μg/mL of IC_{50} and 339.0 ± 0.412 μg/mL of CC_{50} which was almost equal to that of **5m** and thus this compound too can be

Table 3 Screening results of activity of **5a–n** against ovarian cancer cell line.



No.	R	[a]IC_{50} μg/ml ± SD	[b]CC_{50} μg/ml ± SD
		SK-OV-3	MDCK
5a	H	55.94 ± 0.890	306.4 ± 0.886
5b	6-NO_2	55.50 ± 0.415	303.6 ± 0.059
5c	6-COOH	44.58 ± 1.198	308.1 ± 0.410
5d	6-CH_3	47.72 ± 0.334	309.1 ± 0.233
5e	6-OCH_3	37.52 ± 0.469	339.0 ± 0.412
5f	6-OC_2H_5	47.77 ± 1.072	325.9 ± 0.397
5g	6-Cl	44.32 ± 0.303	333.9 ± 0.624
5h	6-Br	50.32 ± 1.170	318.4 ± 1.452
5i	6-I	54.27 ± 0.637	302.8 ± 0.735
5j	6-F	53.46 ± 0.266	308.3 ± 1.271
5k	4,6-di F	52.00 ± 0.603	314.2 ± 0.663
5l	6-CF_3	53.72 ± 1.100	321.2 ± 3.275
5m	6-CN	32.61 ± 0.581	312.8 ± 1.206
5n	–	52.69 ± 1.060	307.0 ± 1.425
Berberine		24.17 ± 1.196	111.8 ± 0.658

[a] Anticancer activities are shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results expressed as an average ± standard deviation.
[b] CC_{50} – cytotoxicity concentration of 50%.

regarded as one of the most potent analogs against SK-OV-3 among all tested. Lastly, a scaffold carrying EWD chlorine atom (**5g**) exhibited 44.32 ± 0.303 μg/mL of IC_{50} and 333.9 ± 0.624 μg/mL of CC_{50} and can be said that it had remarkable sensitivity against SK-OV-3 cell line. Several remaining compounds demonstrated nearly 45–55 μg/mL of IC_{50}, for example, compounds **5c**, **5d**, **5f**, **5h** and **5k** and were regarded to have noticeable effects against ovarian cancer cell line SK-OV-3 because all have yielded reasonable IC50s and tolerable cytotoxicities when compared to that of berberine. All the remaining scaffolds are found to express good to moderate inhibition of SK-OV-3 cell line.

4. Conclusion

In summary, berberine-benzothiazole conjugates were developed to study their antioxidant and anticancer effects. Compounds bearing methoxy or cyano functional groups were the most active DPPH and ABTS radical scavengers. In general, scaffolds carrying EWD groups were more active in the antioxidant assay than those with halogen functional groups. Analogs bearing methyl and cyano functional groups presented the most significant anticancer effects against the HeLa and CaSki cervical cancer cell lines and the SK-OV-3 ovarian cancer cell line. Hence, these results suggest that additional efforts to modify natural product molecules to achieve better pharmacological effects are warranted.

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