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ORIGINAL RESEARCH

Synthesis and anticancer activity of chalcone analogues with sulfonyl groups

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Abstract

Three series of sulfonyl esters were synthesized in reactions of sulfonyl chlorides with three different phenolic chalcone analogues (dehydrozingerone (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one), (*E*)-1-(4-hydroxy-3-methoxyphenyl)pent-1-en-3-one, and (*E*)-1-(4-hydroxy-3-methoxyphenyl)-5-methylhex-1-en-3-one). The structures of the new compounds were determined by IR, MS, and NMR methods. Screening of the new sulfonyl esters' in vitro cytotoxic activities against human epithelial cervical carcinoma (HeLa) and normal human fibroblast (MRC-5) cell lines by the MTT method was performed. The five most active were selected and further tested on HeLa, MRC-5, and MCF-7 (breast carcinoma) cell lines. The examined compounds exhibit strong in vitro anticancer activities with moderate-to-high selectivity, inducing apoptotic cell death and cell cycle arrest in both HeLa and MCF-7 cell lines, but have little to no effect on the non-cancerous MRC-5 cell line.

Keywords Apoptosis · Chalcones · Cell death · Cytotoxicity · Sulfonyl ester

Introduction

Cancer is one of the most human widespread diseases, so there is considerable scientific and commercial interest in discovering new anticancer agents from naturally sourced products (Kinghorn et al. 2003). Many natural products and dietary components have been evaluated as potential chemopreventive agents; herbal remedies used in traditional medicine are a promising source of potentially active drugs (Rajasekar et al. 2012). Although many anticancer drugs of natural origin have been discovered, the search for new anticancer agents is still of great importance, particularly in terms of increasing their bioavailability and finding less toxic and more effective drugs.

Chalcones (*trans*-1,3-diaryl-2-propen-1-ones) are natural products of the flavonoid family; they are considered as intermediates in flavonoid biosynthesis, and are widespread in plants. These compounds are characterized by the presence of two phenyl rings linked by a three-carbon bridge that contains an α,β-unsaturated ketone fragment, forming the central core in a variety of important biological compounds (Mahapatra et al. 2015; Tseng et al. 2015). Chalcones and their derivatives have fascinated chemists due to their numerous useful biological and pharmacological properties, such as antioxidant (Vasil'ev et al. 2010; Sivakumar et al. 2011; Vogel et al. 2008), anti-inflammatory (Yadav et al. 2010; Zhang et al. 2010), antibacterial (Nielsen et al. 2004; Batovska et al. 2009; Alcaradz et al. 2000), antifungal (Lahtchev et al. 2008;

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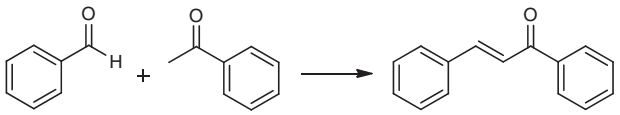


Fig. 1 Synthesis of chalcones

ElSohly et al. 2001; Gafner et al. 1996), antiviral (Trivedi et al. 2007; Phrutivorapongkul et al. 2003; Park et al. 2011), antiparasitic (Nielsen et al. 1995, 1998), anticancer (Zsoldos-Mady et al. 2006; Anto et al. 1995), antileishmanial (Chen et al. 1993, 1994; Liu et al. 2001; Li et al. 1995; Narender et al. 2005), and antitubercular activities (Hans et al. 2010; Sivakumar et al. 2007).

Classical synthesis of chalcones involves the Claisen–Schmidt condensation reaction of aryl methyl ketones with aromatic aldehydes (Fig. 1).

A well-known natural phenolic product, dehydrozingerone (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, **3**), is isolated from ginger root (*Zingiber officinale*), and is a very important chalcone analog, which exhibits anti-inflammatory, antioxidant, and antitumor-promoting activities (Motohashi et al. 1998).

Dehydrozingerone and its semi-chalcone analogs, with alkyl groups linked to the carbonyl groups instead of to the aromatic groups (as in standard chalcones), were prepared. These compounds have well-established biological activities, as was previously reported (Muškinja et al. 2016). Furthermore, the free phenolic groups reacted with corresponding aryl sulfonyl chlorides to form esters (Loewenthal 1959). Although aryl sulfonates are widely used as protective agents for hydroxy groups, they are highly stable and usually require drastic deprotection conditions. Compounds containing aryl sulfonate moieties have received considerable attention during the last two decades, as they are endowed with a variety of biological activities (anti-papillomavirus (Christensen et al. 2001), anti-HIV-1 (Rusconi et al. 1996), antineoplastic (Hanna et al. 1991), as well as having activities against various kinds of cancers (Betts et al. 2006; Cyr et al. 2008)).

Materials and methods

Chemistry

All starting chemicals were commercially available and used as received, except the solvents, which were purified by distillation.

Column chromatography: silica gel 60 (Merck, 230-400 mesh ASTM); TLC: silica gel 60 F_{254}-precoated plates (Merck); IR spectra: Perkin-Elmer Spectrum One FT-IR spectrometer with a KBr disc, ν in cm^{-1}; NMR spectra: Varian Gemini 200 MHz spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C), using $CDCl_3$ as the solvent and TMS as the internal standard. ^{1}H– and ^{13}C–NMR chemical shifts were reported in parts per million (p.p.m.) and were referenced to the solvent peak; $CDCl_3$ (7.26 p.p.m. for ^{1}H and 76.90 p.p.m. for ^{13}C). Multiplicities were represented by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) were in Hertz (Hz).

Mass spectrometry was performed using a Waters Micromass ZQ mass spectrometer and MassLynx software for control and data processing. Electro spray ionization in the positive mode was used. The electro spray capillary was set at 4.3 kV and the cone at 40 V. The ion source temperature was 125 °C, and the nitrogen flow rates were 400 and 50 l/h, for desolvation and cone gas flow, respectively. The collision energy was 40 eV. The melting point of the products was determined using a MelTemp1000 apparatus.

Chemical synthesis

General procedure for synthesis of (*E*)-2-methoxy-4-(3-oxoprop-1-enyl-3-alkyl)phenyl benzenesulfonates

Solutions of selected methyl ketones (2 mmol) in methanol (6 mL) were stirred and corresponding sulfonyl chlorides (2.4 mmol) were added. Then, triethylamine (0.6 mL) was added dropwise. The mixtures were stirred overnight at room temperature. After completion of the reactions, mixtures were poured into iced water with stirring. In some cases (for our compounds **7f**, **7g**, **8e**, **8f**, **8g**, and **9g**; see below), the formed precipitate was filtered and washed with cold diethyl ether. In other cases, when no precipitate formed, the organic layer was extracted with dichloromethane (3 × 50 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was purified by short column chromatography on silica gel, using dichloromethane as eluent. The oil obtained usually crystallized; if not, the oily residue was dissolved in diethyl ether from which products crystallized on standing in a deepfreeze. Crude products were washed with cold ether, and 21 pure, mostly white crystals were obtained, as follows:

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl benzenesulfonate (7a) Yellowish white crystals; yield: 88.7%; m.p. 84–85 °C; IR (KBr): 3075, 3009, 1666, 1626, 1585, 1371, 1198, 1116, 1091, 1031, 861 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.38$ (s, 3H, CH_3CO), 3.56 (s, 3H, OCH_3), 6.63 (d, 1H, $J = 16.2$ Hz, CH), 6.97–7.28 (m, 3H, Ar–H), 7.42 (d, 1H, $J = 16.2$ Hz, CH), 7.49–7.71 (m, 3H, Ar–H), 7.86–7.89 (m, 3H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.5$, 55.6, 111.5, 121.1, 124.5, 127.9, 128.5, 128.8, 134, 134.5, 136.1, 139.7, 141.9, 152, 197.9 (CO). ESI-MS (40 eV): *m/z* (%) = 332.37 (100%) $[M]^+$, 141.17 (17%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-methylbenzenesulfonate (7b) White crystals; yield: 97.4%; m.p. 130–131 °C; IR (KBr): 3010, 2945, 1667, 1626, 1597, 1362, 1149, 1114, 1086, 1032, 856 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.37$ (s, 3H, CH_3CO), 2.45 (s, 3H, CH_3), 3.59 (s, 3H, OCH_3), 6.63 (d, 1H, $J = 16.2$ Hz, CH), 6.99–7.09 (m, 2H, Ar–H), 7.17 (d, 1H, $J = 8.2$ Hz, Ar–H), 7.28–7.33 (m, 2H, Ar–H), 7.42 (d, 1H, $J = 16.4$ Hz, CH), 7.73–7.77 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 21.6$, 27.5, 55.6, 111.6, 121, 124.3, 127.8, 128.5, 129.4, 133.1, 134.3, 139.8, 141.9, 145.2, 152.1, 197.9 (CO). ESI-MS (40 eV): *m/z* (%) = 346.40 (100%) $[M]^+$, 155.19 (31%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-methoxybenzenesulfonate (7c) Yellowish white crystals; yield: 82.2%; m.p. 128–129 °C; IR (KBr): 3091, 3014, 1670, 1645, 1598, 1501, 1357, 1173, 1092, 1024, 852 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.37$ (s, 3H, CH_3CO), 2.62 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.63 (d, 1H, $J = 16.2$ Hz, CH), 6.94–7.29 (m, 5H, Ar–H), 7.42 (d, 1H, $J = 16.4$ Hz, CH), 7.77–7.82 (m, 3H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.5$, 55.6, 55.7, 111.6, 113.9, 121.1, 124.4, 127.4, 127.8, 130.8, 134.3, 139.9, 141.9, 152.1, 164.1, 197.9 (CO). ESI-MS (40 eV): *m/z* (%) = 362.40 (46%) $[M]^+$, 171.19 (100%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-tert-butylbenzenesulfonate (7d) Yellowish white crystals; yield: 92%; m. p. 92–94 °C; IR (KBr): 3079, 2964, 1689, 1609, 1507, 1370, 1154, 1109, 1087, 1028, 852 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.35$ (s, 9H, $(CH_3)_3$–C), 2.38 (s, 3H, CH_3CO), 3.54 (s, 3H, OCH_3), 6.64 (d, 1H, $J = 16.2$ Hz, CH), 6.97–7.28 (m, 3H, Ar–H), 7.43 (d, 1H, $J = 16.4$ Hz, CH), 7.53 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.79 (d, 2H, $J = 8.8$ Hz, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.5$, 30.9, 35.3, 55.5, 111.4, 121.1, 124.6, 125.7, 127.8, 128.4, 133, 134.4, 139.8, 141.9, 152.1, 158.2, 197.9 (CO). ESI-MS (40 eV): *m/z* (%) = 388.48 (100%) $[M]^+$, 197.27 (39%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-bromobenzenesulfonate (7e) Yellowish crystals; yield: 71.9%; m.p. 123-124 °C; IR (KBr): 3090, 2945, 1676, 1655, 1571, 1374, 1199, 1168, 1113, 1067, 831 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.38$ (s, 3H, CH_3CO), 3.59 (s, 3H, OCH_3), 6.64 (d, 1H, $J = 16.2$ Hz, CH), 6.99–7.28 (m, 3H, Ar–H), 7.42 (d, 1H, $J = 16.4$ Hz, CH), 7.64–7.76 (m, 4H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.6$, 55.6, 111.6, 121.1, 124.5, 128, 129.4, 129.9, 132.1, 134.7, 135.1, 139.5, 141.7, 151.8, 197.8 (CO). ESI-MS (40 eV): *m/z* (%) = 411.27 (29%) $[M]^+$, 220.06 (4%), 155.19 (100%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-chlorobenzenesulfonate (7f) Yellowish powder; yield: 88%; m.p. 110–112 °C; IR (KBr): 3091, 2985, 1667, 1641, 1586, 1372, 1194, 1114, 1091, 1027, 857 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.38$ (s, 3H, CH_3CO), 3.59 (s, 3H, OCH_3), 6.64 (d, 1H, $J = 16.4$ Hz, CH), 6.99–7.28 (m, 3H, Ar–H), 7.42 (d, 1H, $J = 16.4$ Hz, CH), 7.48–7.52 (m, 2H, Ar–H), 7.79–7.84 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.6$, 55.5, 111.6, 121.1, 124.5, 128, 129.1, 129.9, 134.5, 134.6, 139.5, 140.8, 141.7, 151.8, 197.8 (CO). ESI-MS (40 eV): *m/z* (%) = 366.82 (100%) $[M]^+$, 175.61 (36%).

(*E*)-2-methoxy-4-(3-oxobut-1-enyl)phenyl 4-nitrobenzene sulfonate (7g) Light beige powder; yield: 83.8%; m.p. 149–151 °C; IR (KBr): 3113, 2979, 1674, 1651, 1590, 1376, 1350, 1199, 1112, 1085, 1029, 842 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 2.38$ (s, 3H, CH_3CO), 3.59 (s, 3H, OCH_3), 6.65 (d, 1H, $J = 16.2$ Hz, CH), 7.00–7.28 (m, 3H, Ar–H), 8.10 (d, 2H, $J = 8.8$ Hz, Ar–H), 8.38 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.42 (d, 1H, $J = 16.2$ Hz, CH); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 27.6$, 55.6, 111.7, 121.2, 123.9, 124.5, 128.3, 129.9, 135.1, 139.2, 141.5, 141.8, 150.9, 151.6, 197.7 (CO). ESI-MS (40 eV): *m/z* (%) = 377.37 (100%) $[M]^+$, 186.17 (7%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl benzenesulfonate (8a) Yellowish crystals; yield: 93.1%; m.p. 80–82 °C; IR (KBr): 2979, 2876, 1669, 1629, 1400, 1287, 1188, 1113, 1088, 1035, 867 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta =$ 1.16 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 2.69 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.56 (s, 3H, OCH_3), 6.66 (d, 1H, $J = 16.2$ Hz, CH), 6.97–7.28 (m, 3H, Ar–H), 7.42–7.67 (m, 4H, CH, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 7.85–7.90 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 34.1, 55.6, 111.6, 120.9, 124.4, 126.8, 128.5, 128.8, 134, 134.6, 136.1, 139.6, 140.8, 151.9, 200.4 (CO). ESI-MS (40 eV): *m/z* (%) = 346.40 (100%) $[M]^+$, 141.17 (24%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-methylbenzenesulfonate (8b) Yellowish white crystals; yield: 90.5%; m.p. 72–73 °C; IR (KBr): 2968, 2935, 1689, 1612, 1598, 1369, 1177, 1119, 1090, 1032, 851 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.16$ (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 2.45 (s, 3H, CH_3), 2.69 (q, 2H, $J = 7.2$ Hz, CH_3CH_2), 3.60 (s, 3H, OCH_3), 6.66 (d, 1H, $J = 16.2$ Hz, CH), 6.98–7.33 (m, 5H, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 7.73–7.79 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 21.6, 34.1, 55.6, 111.7, 120.9, 124.4, 126.8, 128.6, 129.4, 133.2, 134.5, 139.8, 140.8, 145.2, 152.1, 200.5 (CO). ESI-MS (40 eV): *m/z* (%) = 360.42 (100%) $[M]^+$, 155.19 (44%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-methoxybenzenesulfonate (8c) Yellowish white crystals; yield: 83.1%; m.p. 108–109 °C; IR (KBr): 2982, 1674, 1585, 1366, 1191, 1117, 1088, 1025, 833 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta =$ 1.16 (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 2.69 (q, 2H, $J = 7.2$ Hz,

CH_3CH_2), 3.62 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.66 (d, 1H, $J = 16.2$ Hz, CH), 6.94–7.28 (m, 5H, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 7.75–7.83 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 34.1, 55.6, 55.7, 111.6, 113.9, 120.9, 124.4, 126.7, 127.4, 130.8, 134.5, 139.8, 140.8, 152.1, 164, 200.4 (CO). ESI-MS (40 eV): *m/z* (%) = 376.42 (58%) $[M]^+$, 171.19 (100%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-(tert-butyl)benzenesulfonate (8d) White crystals; yield: 78.7%; m.p. 120–121 °C; IR (KBr): 2972, 1662, 1595, 1370, 1190, 1110, 1087, 1030, 853 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.16$ (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 1.35 (s, 9H, 3xCH_3), 2.69 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.54 (s, 3H, OCH_3), 6.67 (d, 1H, $J = 16.2$ Hz, CH), 6.97–7.28 (m, 3H, Ar–H), 7.43–7.56 (m, 3H, CH, Ar–H), 7.77–7.81 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 30.9, 34.1, 35.3, 55.5, 111.5, 120.9, 124.6, 125.8, 126.8, 128.4, 133, 134.5, 139.7, 140.8, 152, 158.1, 200.5 (CO). ESI-MS (40 eV): *m/z* (%) = 402.49 (100%) $[M]^+$, 346.38 (14%), 197.27 (37%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-bromobenzenesulfonate (8e) White powder; yield: 84.2%; m.p. 110–111 °C; IR (KBr): 2984, 1668, 1587, 1375, 1176, 1115, 1085, 1027, 830 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.16$ (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 2.69 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.59 (s, 3H, OCH_3), 6.67 (d, 1H, $J = 16.2$ Hz, CH), 6.98–7.27 (m, 3H, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 7.64–7.76 (m, 4H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 34.2, 55.6, 111.6, 121, 124.5, 126.9, 129.4, 130, 132.1, 134.9, 135.1, 139.4, 140.6, 151.8, 200.4 (CO). ESI-MS (40 eV): *m/z* (%) = 425.29 (24%) $[M]^+$, 220.06 (4%), 175.59 (33%), 155.18 (100%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-chlorobenzenesulfonate (8f) White powder; yield: 87.9%; m.p. 122–124 °C; IR (KBr): 2972, 1667, 1629, 1585, 1375, 1198, 1115, 1091, 1024, 845 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.16$ (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 2.69 (q, 2H, $J = 7.2$ Hz, CH_3CH_2), 3.60 (s, 3H, OCH_3), 6.67 (d, 1H, $J = 16.0$ Hz, CH), 6.99–7.27 (m, 3H, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 7.42–7.52 (m, 2H, Ar–H), 7.78–7.84 (m, 2H, Ar–H);^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8.1$, 34.2, 55.6, 111.6, 121, 124.5, 126.9, 129.1, 129.9, 134.6, 134.9, 139.4, 140.6, 140.8, 151.8, 200.4 (CO). ESI-MS (40 eV): *m/z* (%) = 380.84 (90%) $[M]^+$, 175.61 (100%).

(*E*)-2-methoxy-4-(3-oxopent-1-enyl)phenyl 4-nitrobenzenesulfonate (8g) Light beige powder; yield: 85.7%; m.p. 140–141 °C; IR (KBr): 2978, 2877, 1662, 1589, 1536, 1379, 1199, 1188, 1111, 1089, 1024, 848 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 1.16$ (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 2.69 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.59 (s, 3H, OCH_3), 6.68 (d, 1H, $J = 16.2$ Hz, CH), 7.00–7.28 (m, 3H, Ar–H), 7.46 (d, 1H, $J = 16.2$ Hz, CH), 8.07–8.12 (m, 2H, Ar–H), 8.35–8.40 (m, 2H, Ar–H);^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 8$, 34.2, 55.6, 111.8, 121.1, 123.9, 124.4, 127.2, 129.9, 135.2, 139.1, 140.3, 141.8, 150.9, 151.6, 200.3 (CO). ESI-MS (40 eV): *m/z* (%) = 391.40 (100%) $[M]^+$, 186.17 (18%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl benzenesulfonate (9a) Yellow crystals; yield: 96.2%; m.p. 59–61 °C; IR (KBr): 2952, 2869, 1658, 1618, 1598, 1369, 1188, 1116, 1089, 1036, 841 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.97$ (d, 6H, $J = 6.8$ Hz, 2xCH_3), 2.15–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.56 (s, 3H, OCH_3), 6.65 (d, 1H, $J = 16.2$ Hz, CH), 6.97–7.27 (m, 3H, Ar–H), 7.44 (d, 1H, $J = 16.2$ Hz, CH), 7.52–7.67 (m, 3H, Ar–H), 7.86–7.89 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 22.6$, 25.1, 49.9, 55.6, 111.6, 120.9, 124.5, 128.5, 128.8, 134, 134.6, 136.1, 139.6, 140.9, 151.9, 199.9 (CO). ESI-MS (40 eV): *m/z* (%) = 374.46 (100%) $[M]^+$, 141.17 (36%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-methylbenzenesulfonate (9b) Yellowish powder; yield: 87.9%; m.p. 96–97 °C; IR (KBr): 2958, 2873, 1687, 1612, 1598, 1370, 1177, 1064, 1030, 856 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.97$ (d, 6H, $J = 6.8$ Hz, 2xCH_3), 2.16–2.29 (m, 1H, CH), 2.45 (s, 3H, CH_3), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.60 (s, 3H, OCH_3), 6.65 (d, 1H, $J = 16.2$ Hz, CH), 6.98–7.33 (m, 5H, Ar–H), 7.44 (d, 1H, $J = 16.2$ Hz, CH), 7.73–7.78 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 21.6$, 22.6, 25.1, 49.9, 55.6, 111.7, 120.9, 124.4, 127.4, 128.5, 129.4, 133.1, 134.5, 139.8, 141, 145.2, 152.1, 199.9 (CO). ESI-MS (40 eV): *m/z* (%) = 388.48 (100%) $[M]^+$, 155.19 (79%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-methoxybenzenesulfonate (9c) White crystals; yield: 88.5%; m.p. 74–75 °C; IR (KBr): 2954, 2873, 1687, 1610, 1597, 1368, 1170, 1094, 1030, 856 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.97$ (d, 6H, $J = 6.6$ Hz, 2xCH_3), 2.15–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 6.8$ Hz, CH_2), 3.62 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.65 (d, 1H, $J = 16.2$ Hz, CH), 6.94–7.27 (m, 5H, Ar–H), 7.44 (d, 1H, $J = 16.2$ Hz, CH), 7.77–7.82 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 21.6$, 22.6, 25.1, 49.9, 55.6, 111.7, 120.9, 124.4, 127.4, 128.5, 129.4, 133.1, 134.5, 139.8, 141, 145.2, 152.1, 199.9 (CO). ESI-MS (40 eV): *m/z* (%) = 404.48 (44%) $[M]^+$, 171.19 (100%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-tert-butylbenzenesulfonate (9d) Yellowish crystals; yield: 86.9%; m.p. 86–87 °C; IR (KBr): 2957, 2900, 1658, 1628,

1596, 1370, 1179, 1111, 1087, 1032, 859 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.97$ (d, 6H, $J = 6.6$ Hz, 2xCH_3), 1.35 (s, 9H, 3xCH_3), 2.16–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.54 (s, 3H, OCH_3), 6.66 (d, 1H, $J =$ 16.2 Hz, CH), 6.97–7.28 (m, 3H, Ar–H), 7.45 (d, 1H, $J =$ 16.2 Hz, CH), 7.50–7.55 (m, 2H, Ar–H), 7.77–7.81 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 22.6$, 25.1, 30.9, 35.3, 49.9, 55.5, 111.5, 120.9, 124.6, 125.8, 127.3, 128.4, 133, 134.5, 139.7, 141, 152, 158.1, 199.9 (CO). ESI-MS (40 eV): *m/z* (%) = 430.56 (100%) $[M]^+$, 197.27 (96%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-brom obenzenesulfonate (9e) Yellowish powder; yield: 83.9%; m.p. 114–115 °C; IR (KBr): 2952, 2868, 1659, 1598, 1586, 1377, 1187, 1115, 1086, 1033, 839 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.98$ (d, 6H, $J = 6.6$ Hz, 2xCH_3), 2.16–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.59 (s, 3H, OCH_3), 6.66 (d, 1H, $J = 16.2$ Hz, CH), 6.98–7.27 (m, 3H, Ar–H), 7.44 (d, 1H, $J = 16.2$ Hz, CH), 7.64–7.76 (m, 4H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 22.6$, 25.1, 50, 55.6, 111.7, 121.1, 124.5, 127.6, 129.4, 130, 132.1, 134.9, 135.1, 139.4, 140.8, 151.8, 199.8 (CO). ESI-MS (40 eV): *m/z* (%) = 453.35 (21%) $[M]^+$, 220.07 (6%), 155.18 (100%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-chlor obenzenesulfonate (9f) Yellowish crystals; yield: 90.9%; m.p. 115-117 °C; IR (KBr): 2956, 2871, 1659, 1621, 1585, 1377, 1187, 1116, 1090, 1033, 844 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.98$ (d, 6H, $J = 6.6$ Hz, 2xCH_3), 2.16–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.59 (s, 3H, OCH_3), 6.66 (d, 1H, $J = 16.2$ Hz, CH), 6.99–7.27 (m, 3H, Ar–H), 7.40–7.52 (m, 3H, CH, Ar–H), 7.79–7.84 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 22.6$, 25.1, 50, 55.6, 111.7, 121, 124.5, 127.6, 129.1, 129.9, 134.6, 134.9, 139.4, 140.7, 140.8, 151.8, 199.8 (CO). ESI-MS (40 eV): *m/z* (%) = 408.9 (59%) $[M]^+$, 175.61 (100%).

(*E*)-2-methoxy-4-(5-methyl-3-oxohex-1-enyl)phenyl 4-nitrob enzenesulfonate (9g) Beige powder; yield: 80.5%; m.p. 138–139 °C; IR (KBr): 2962, 2870, 1664, 1540, 1378, 1186, 1113, 1089, 1025, 848 cm^{-1}; ^{1}H–NMR (200 MHz, $CDCl_3$): $\delta = 0.97$ (d, 6H, $J = 6.8$ Hz, 2xCH_3), 2.16–2.29 (m, 1H, CH), 2.53 (d, 2H, $J = 7.0$ Hz, CH_2), 3.59 (s, 3H, OCH_3), 6.67 (d, 1H, $J = 16.2$ Hz, CH), 7.00–7.28 (m, 3H, Ar–H), 7.44 (d, 1H, $J = 16.2$ Hz, CH), 8.08–8.12 (m, 2H, Ar–H), 8.36–8.40 (m, 2H, Ar–H); ^{13}C–NMR (50 MHz, $CDCl_3$): $\delta = 22.5$, 25, 48.9, 55.5, 111.7, 121.1, 123.8, 124.4, 127.7, 129.8, 135.2, 139, 140.4, 141.8, 150.8, 151.5, 199.6 (CO). ESI-MS (40 eV): *m/z* (%) = 419.45 (56%) $[M]^+$, 186.17 (47%).

Cytotoxicity

Cell cultures

Epithelial cervical carcinoma (HeLa), breast carcinoma (MCF-7), and the normal human fibroblast (MRC-5) cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (all from Sigma, Germany). Antibiotics were omitted from the medium to avoid undesired interactions. The cells were cultivated at 37 °C in absolute humidity in an atmosphere containing 5% CO_2 and were subcultured two to three times a week.

Preparation of sulfonyl ester and control solutions

All compounds examined for cytotoxicity, including cisplatin (used as a control), were dissolved in dimethyl sulfoxide (DMSO; Fisher Scientific, UK) at a concentration of 20 mM, filtered through 0.22 mm Millipore filters and diluted appropriately with DMEM to working concentrations of 0.3, 1, 3, 10, 30, and 100 µM. The final concentration of DMSO in DMEM never exceeded 0.5% (vol/vol). All solutions were freshly prepared on the day of cell line treatment.

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of the compounds as previously described (Zaric et al. 2015). The cells were harvested from the culture flasks during the exponential growth phase, counted and 5×10^3 cells per well were seeded into 96-well culture plates. The cells were allowed to adhere overnight in a humidified incubator with 5% CO_2. Afterwards, the supernatants were removed and the remaining cell monolayers were treated with 200 µL volumes of dilutions of the investigated compounds in fresh DMEM, as described above. Fresh DMEM was used as a control. A positive control containing cisplatin was used as reference drug for comparison. Controls wells were treated the same as test wells. All cells were incubated at 37 °C in an atmosphere of 5% CO_2 and absolute humidity for 24 and 48 h. Then, the cell culture media (with the investigated compounds) was removed, and 100 µL of MTT (0.5 mg/mL) was added to each well. After 2 h incubation at 37 °C, MTT solution was removed, and 150 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured with a multiplate reader (Zenyth 3100, Anthos Labtec Instruments GmbH, Austria) at 595 nm.

All compounds were examined in triplicate and repeated in three independent series. The percentage of cytotoxicity was calculated using the formula: $((A_{control} - A_{test})/A_{control}) \times 100$, where A is the absorbance determined for untreated (control) and treated (test) cells. The selectivity indexes (SI) for all examined compounds after 48 h screening were calculated using the formula: SI = (% cytotoxicity HeLa/% cytotoxicity MRC-5) × % cytotoxicity HeLa. Also, using Microsoft Office Excel 2010, 50% inhibitory concentration (IC_{50}), i.e., the concentration required to cause toxic effects in 50% of intact cells, was calculated. Selectivity index (SI) values to identify selective anticancer cell activity were calculated by dividing the IC_{50} values of normal MRC-5 cells by the IC_{50} of HeLa cells (Joksimović et al. 2016).

Flow cytometric analysis

The Annexin V-FITC/7-AAD kit (Beckman Coulter, USA) was used according to the manufacturer's instructions to determine the type of cell death induced by the examined compounds. Briefly, HeLa and MCF-7 cells were treated with the examined compounds in concentrations corresponding to their IC_{50} values for 48 h at 37 °C in an atmosphere of 5% CO_2 and absolute humidity. The control was DMEM alone, treated the same as the examined compounds. Then, cells were collected, washed in phosphate buffered saline (PBS), and finally suspended in ice-cold binding buffer (1×10^5 cells/100 µL binding buffer). Cells were stained with 10 µL of Annexin V-FITC and 20 µL 7-AAD, and after 15 min' incubation in the dark, 400 µL of binding buffer was added. Cells were analyzed using a flow cytometer, Cytomics FC500 (Beckman Coulter, USA), and the percentages of viable, apoptotic, and necrotic cells were evaluated using Flowing Software (http://www.flowingsoftware.com/). The results were presented as dot plots.

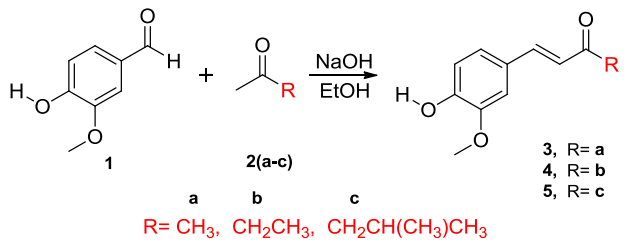
Cell cycle analysis

HeLa and MCF-7 cells were treated with the examined compounds in concentrations corresponding to their IC_{50} values for 48 h at 37 °C in an atmosphere of 5% CO_2 and absolute humidity. The control was DMEM alone, treated the same as the examined compounds. After incubation, cells were collected into tubes, washed in PBS, and finally suspended in 1 mL of ice-cold 70% ethanol. After overnight incubation at 4 °C, cells were washed in PBS and treated with RNase A (500 µg/mL PBS) for 30 min at 37 °C. Propidium iodide (5 µL of 10 mg/mL PBS) was added to each tube, and after 15 min' incubation in the dark, cells were assayed using a flow cytometer Cytomics FC500. The data were analyzed using FlowJo Software and the results were presented as histograms.

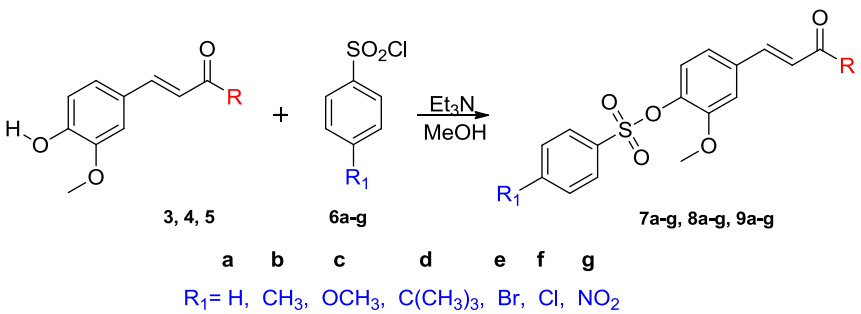
Results and discussion

Chemical synthesis

In continuation of our work on the synthesis of chalcone analogs, we decided to synthesize some new compounds with various sulfonyl groups and vanillin' fragments incorporated in the same molecule, starting from the easily accessible, natural product, vanillin. In the first step, using Claisen–Schmidt condensation, vanillin (**1**) was condensed with three different non-aromatic methyl ketones (acetone (**2a**), 4-methyl-2-pentanone (**2b**), and 2-butanone (**2c**)). These condensation reactions produced chalcone analogs **3**, **4**, and **5**, according to Scheme 1. Chalcone analog **3** was synthesized following a previously published procedure (Smith 1996), whereas chalcone analogs **4** and **5** were synthesized following a different method (Muškinja et al. 2016).

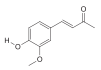
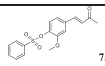
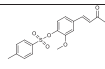
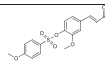
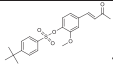
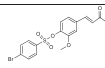
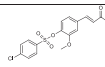
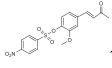
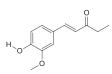
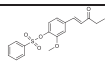
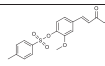
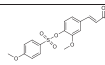
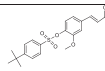
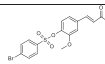
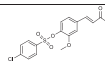
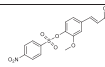
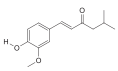
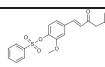
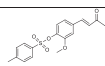
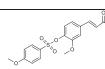
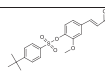
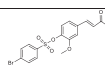
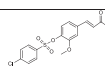
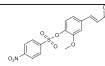


Scheme 1 Synthesis of chalcone analogs **3**, **4**, and **5** from vanillin **1**



Scheme 2 Synthesis of sulfonyl esters **7**, **8**, and **9** in reactions of chalcone analogs with sulfonyl chlorides **6a–g**

Table 1 Synthesized sulfonyl esters

Entry	Chalcone analogues	Sulfonyl chloride	Product	Yield (%)
1		**6a**	 **7a**	88.7
		6b	 **7b**	97.4
		6c	 **7c**	82.2
		6d	 **7d**	92
		6e	 **7e**	71.9
		6f	 **7f**	88
		6g	 **7g**	83.8
2		**6a**	 **8a**	93.1
		6b	 **8b**	90.5
		6c	 **8c**	83.1
		6d	 **8d**	78.7
		6e	 **8e**	84.2
		6f	 **8f**	87.9
		6g	 **8g**	85.7
3		**6a**	 **9a**	96.2
		6b	 **9b**	87.9
		6c	 **9c**	88.5
		6d	 **9d**	86.9
		6e	 **9e**	83.9
		6f	 **9f**	90.9
		6g	 **9g**	80.5

Synthesized chalcone analogs were used as substrates for further transformations. In reactions of their free phenolic groups with sulfonyl chlorides in ethanolic solution, triethylamine was used as a base, and corresponding sulfonyl esters **7a–g**, **8a–g**, and **9a–g** were obtained (Scheme 2). These reactions produced very high yields of sulfonyl esters (Table 1), and the products were characterized by their spectral data (IR, ^{1}H–NMR and ^{13}C–NMR). The aim of this study was to obtain compounds which had enone systems and sulfonyl groups, as these groups are mostly responsible for cytotoxic activity against different cell lines.

Cytotoxic activities

Sulfonyl esters 7g, 8b, 8e, 9a, and 9g demonstrate high cytotoxicity against tumor cell lines

The cytotoxic activities of the 21 examined sulfonyl esters from three groups (**7a–g**, **8a–g**, and **9a–g**) and cisplatin (each at 100 µM concentration) against HeLa cancer cells and MRC-5 non-cancerous cells were screened after 48 h using the MTT assay (Fig. 2). Sulfonyl esters **8c** and **9b–e** displayed very low cytotoxic activity against HeLa cells. These compounds (at concentrations of 100 µM) were toxic to <70% of cancer cells after 48 h, and therefore, they were not eligible candidates for further investigation. All other examined compounds (100 µM) had very high cytotoxic activity, i.e., up to 96%. Additionally, we screened the cytotoxic activity of the sulfonyl esters against non-cancerous MRC-5 cells, and calculated the selectivity index (SI), in order to select the most effective sulfonyl esters that, at the same time, had activity against cancer cells but were the least damaging to non-cancerous cells.

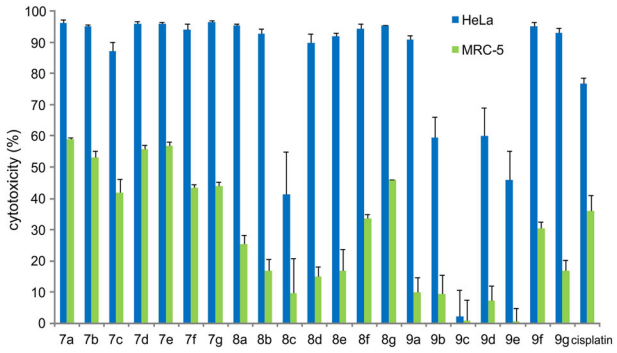


Fig. 2 Cytotoxicity of 21 sulfonyl esters and cisplatin at 100 µM concentrations against HeLa and MRC-5 cells after 48 h. The data show the percentage of cytotoxicity and are means of three independent replicates

Among compounds **7a–g**, sulfonyl ester **7g** had the highest SI (Table S1, supplementary materials). Also, among the other two groups of sulfonyl esters, compounds **8b**, **8e**, **8d**, **9a**, and **9g** displayed very high selective cytotoxicity against cancer cells as demonstrated by their SI values. Therefore, we decided to perform a more detailed analysis of the cytotoxic activity of these six compounds and to calculate their IC_{50} values.

All six examined sulfonyl esters (**7g**, **8b**, **8e**, **8d**, **9a** and **9g**) showed dose-dependent cytotoxic activity toward cancer cells after 24 and 48 h (the results of MTT assay after 24 and 48 h are shown in Fig. S1, supplementary materials). Their IC_{50} values against cancer cell lines (HeLa and MCF-7) and normal human fibroblast MRC-5 cells are shown in Table 2. All compounds showed high cytotoxic activity against cancer cells lines and moderate to low or no cytotoxicity against non-cancerous cells.

Compound **7g** displayed very low selectivity and high cytotoxic activity against both normal and cancer cells. Compound **8e** had the highest cytotoxicity and was four times more selective towards MCF-7 cancer cells than cisplatin, **8b** and **8d**, with an IC_{50} value of 12.5 ± 1.3 µM and SI of 8. Also, compound **9a** was the most cytotoxic and the most selective of all the examined compounds against HeLa cells, with an IC_{50} value of 11.3 ± 0.8 µM and SI of 8.9 after

Table 2 Cytotoxicity (IC_{50}[a] in µM) of six sulfonyl esters and cisplatin against HeLa, MCF-7, and MRC-5 cells and selectivity index (SI) values[b]

Compound	HeLa			MCF-7			MRC-5	
	IC_{50} (24 h)	IC_{50} (48 h)	SI (48 h)	IC_{50} (24 h)	IC_{50} (48 h)	SI (48 h)	IC_{50} (24 h)	IC_{50} (48 h)
7g	58.8 ± 4.2	11.6 ± 0.9	1.6	44.7 ± 2.7	18.4 ± 0.9	1	>100	18.7 ± 2.1
8b	13.8 ± 0.6	14.5 ± 1.3	3.9	84.4 ± 5.5	30.6 ± 1.9	1.9	71.4 ± 5.2	57.1 ± 3.3
8d	43.9 ± 2.7	14 ± 1.1	7.1	91.1 ± 7.1	21.5 ± 1.6	4.7	>100	>100
8e	98.7 ± 6.3	56.9 ± 4.2	1.7	>100	12.5 ± 1.3	8	>100	>100
9a	13.5 ± 0.9	11.3 ± 0.8	8.9	>100	18.1 ± 1.2	5.5	>100	>100
9g	>100	38.6 ± 4.2	2.6	>100	41.5 ± 2.7	2.4	>100	>100
Cisplatin[c]	25.7 ± 1.8	8.7 ± 1	4.9	94.1 ± 8.3	24.5 ± 2.2	1.7	>100	43.1 ± 3.8

[a]IC_{50} is defined as the concentration that causes a 50% cell proliferation inhibition

[b]The data represent the mean ± SD of three independent replicates

[c]Used as a positive control

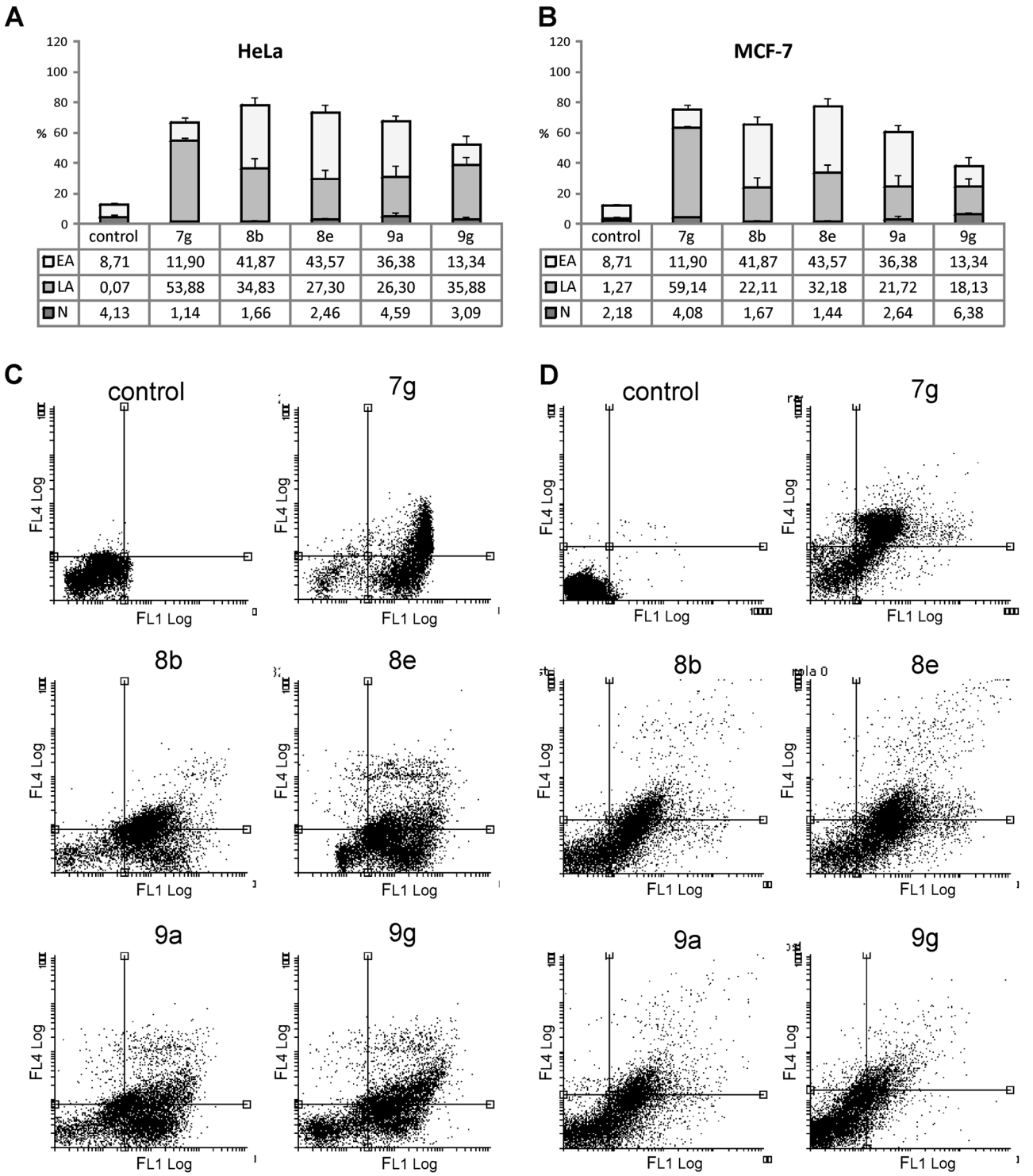


Fig. 3 Flow cytometric analysis of Annexin V-FITC/7-AAD staining. Graphs show percentages of early apoptotic (EA), late apoptotic (LA), and necrotic cells (N) in untreated (control) and treated HeLa **a** and MCF-7 **b** cells. Results are means ± SD of three independent replicates. Dot plots (HeLa **c** and MCF-7 **d**) are representative of two independent analyses

48 h. Furthermore, compounds **7g**, **8e**, and **9a** showed higher cytotoxic activity against MCF-7 cells than did cisplatin under the same conditions. Also, cisplatin displayed moderate cytotoxicity against normal MRC-5 cells, with an IC_{50} value of 43.1 ± 3.8 μM. In contrast to cisplatin, compounds **8d**, **8e**, **9a**, and **9g** had no cytotoxic activity against normal MRC-5 cells with IC_{50} values > 100 μM. Therefore, even though these sulfonyl esters, particularly **8d** and **9a**,

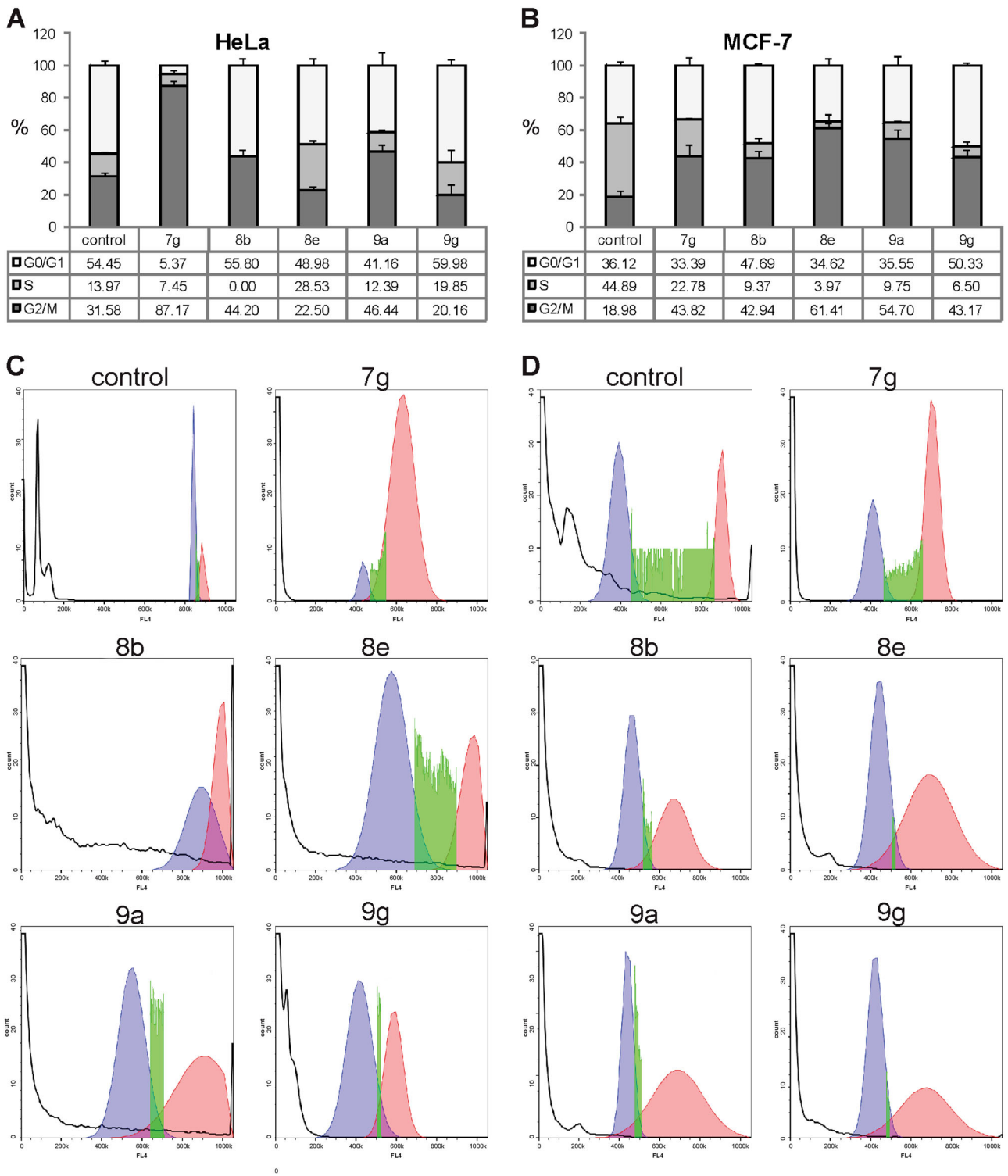


Fig. 4 Cell cycle analysis. Graphs show cell cycle distribution in untreated (control) and treated HeLa **a** and MCF-7 **b** cells. Results are the average of three independent replicates. Histograms (HeLa **c** and MCF-7 **d**) are representative of three independent analyses

had higher IC_{50} values against HeLa cells than cisplatin, they might have promising roles in the treatment of human epithelial cervical carcinoma considering their highly selective cytotoxicity toward cancer cells only.

Our synthesized sulfonyl esters were more potent anticancer agents than similar sulfonamide derivatives described previously (Ghorab et al. 2015). Esters **7g**, **8b**, **8d**, and **9a** showed high cytotoxic activity toward cancer HeLa cells

with IC_{50} values < 15 μM, and esters **7g**, **8e**, and **9a** also showed high anticancer activity toward MCF-7 cancer cells with IC_{50} values < 19 μM. Also, these sulfonyl esters had no cytotoxic activity toward normal MRC-5 cells. Therefore, the examined sulfonyl esters synthesized in the current study might be better candidates for future anticancer research compared to sulfonamide compounds researched earlier.

Benzofuran-substituted chalcone derivates (Coskun et al. 2017) and our newly synthesized chalcone derivates **7g**, **8b**, **8e**, **8d**, **9a**, and **9g** had similar anticancer activities against MCF-7 cancer cells. The cytotoxic activity of benzofuran-substituted chalcone against normal cells has not been reported, to our knowledge. On the other hand, compounds **8d**, **8e**, **9a**, and **9g** had no cytotoxic activity against normal MRC-5 cells.

Based on the results obtained, showing compounds **7g**, **8b**, **8e**, **9a**, and **9g** had high cytotoxicity and the best selectivity (Table 2), we examined the compounds' effects with regard to the predominant type of cell death they caused and to the cell cycle progression of HeLa and MCF-7 cells.

Sulfonyl esters 7g, 8b, 8e, 9a, and 9g induce apoptosis and cell cycle arrest in HeLa and MCF-7 cells

Flow cytometric analysis showed the five examined sulfonyl esters, at concentrations corresponding to their IC_{50} values, induced apoptosis in both HeLa and MCF-7 cells (Fig. 3). The majority of nonviable cells were early or late apoptotic, whereas only minor percentages of cells were necrotic. While cell death due to necrosis induces the inflammatory response and, therefore, injury of surrounding tissue, apoptosis is limited to the dying cell and cause no damage to the neighboring cells. Hence, induction of apoptosis in cancer cells is the main objective of anticancer therapy. However, in cancer cells, the apoptotic machinery is deregulated. Alterations of both extrinsic and intrinsic apoptosis pathways and disturbance of the ratio of proapoptotic and antiapoptotic molecules facilitate survival of cancer cells and tumor formation and progression (Pistritto et al. 2016).

Therefore, the efficacy of antitumor therapy depends on the ability of the therapeutic agent to induce apoptosis. Except for the direct cytotoxic action of antitumor drugs, their cytostatic effects are also advantageous. It should be emphasized that in cancer cells, mechanisms that regulate cell cycle progression are often dysfunctional (Shapiro and Harper 1999). In normal cells, defects emerging from extracellular or intracellular insults result in arrest of cell cycle progression, giving repair mechanisms time to fix the defect. If the damage is irreparable, the damaged cell must be removed, and so the apoptotic program is activated. Conversely, in cancer cells, altered regulatory molecules allow cells to proceed through the cell cycle and proliferate, despite cellular damage. Altered apoptosis and dysfunctional cell cycle regulation give cancer cells a survival advantage, irrespective of intended lifespan, absence of survival factors or presence of injury.

The results of our study showed that all five examined sulfonyl esters induced perturbance in the cell cycles of both cancer cell lines. Given that the selected cell lines have different characteristics and bear mutations in distinctive DNA regions, it can be presumed that different substances have differing effects on the two cancer cell lines. In HeLa cells, sulfonyl esters **7g**, **8b**, and **9a** arrested cells in the G2/M phase, while treatment with **8e** and **9g** resulted in increased percentages of cells in the S phase of the cell cycle. In MCF-7 cells, sulfonyl esters **7g**, **8e**, and **9a** induced G2/M arrest, whereas esters **8b** and **9g** induced higher percentages of cells in both G0/G1 and G2/M phases, indicating impediment of DNA synthesis and cell division (Fig. 4). Altogether, experimental results showed that all examined sulfonyl esters were both cytotoxic and cytostatic towards HeLa and MCF-7 cell lines, and therefore, these novel compounds should be seriously considered as potential anticancer agents.

Conclusion

Three chalcone analogs, starting from the natural product, vanillin, were prepared. The free phenolic groups in these compounds were modified by simple synthesis procedures, giving very high yields of sulfonyl esters. These esters show high in vitro anticancer activity, inducing apoptosis in human epithelial carcinoma HeLa cells and breast carcinoma MCF-7 cell lines. Importantly, these new sulfonyl esters show low or no cytotoxicity against a human fetal lung fibroblast cell line (MRC-5). Also, the examined compounds exert cytostatic action, inducing cell cycle arrest in HeLa and MCF-7 cells. The novel sulfonyl esters can be considered as a significant starting point for the development of new anticancer drugs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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