Synthesis and biological activity of novel series of 4-methoxy, and 4,9-dimethoxy-5-substituted furo[2,3-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives

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ABSTRACT

A novel series of 4-methoxy, and 4,9-dimethoxy-5-substituted furo[2,3-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives 3a,b, 10a–g and 11a–g were prepared in good yields via the reaction of 4-methoxy (1a) and 4,7-dimethoxy-5-acetyl-6-hydroxybenzofurans (1b) and their α,β-unsaturated keto derivatives 6a–g and 7a–g with chlorosulfonyl isocyanate (CSI). On the other hand, N-chlorosulfonyl carbamate derivatives 4a,b, 12a,b and 13a,b were prepared and allowed to react with piperidine to give the corresponding N-piperidinosulfonyl carbamate derivatives 5a,b, 14a,b and 15a,b, respectively. Sixteen new target compounds 3a,b, 10a–g, and 11a–g were tested for their DPPH radical-scavenging, and in vitro antiproliferative activity against A-549, MCF7 and HCT-116 cancer cell lines. Compounds 10a, 11c, 11e, and 11g showed moderate DPPH radical-scavenging activity compared to ascorbic acid at 100 μg/mL. 4,9-Dimethoxy-5-substituted styryl[furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxides 11a, 11b, and 11c were found to be highly active against A-549 and HCT-116 cancer cell lines with IC_{50} values ranging from 0.02 to 0.08 μmol/mL compared to doxorubicin with IC_{50} = 0.04 and 0.06 μmol/mL, respectively.

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Introduction

Chlorosulfonyl isocyanate (CSI) is a remarkable reagent of exceptional reactivity [1,2]. This reagent as a unipartite electrophile and as a versatile heterocumulene is useful in many synthetic transformations and in the synthesis of several heterocyclic systems [3–7]. CSI undergoes nucleophilic addition reaction with salicylaldehydes, 2-hydroxybenzophenones, and 2-hydroxychalcones in dry benzene at 0–5 °C to afford...
N-chlorosulfonyl carbamate derivatives, whereas cycloaddition of CSI with the above moieties in dry toluene at 100 °C produces 1,2,3-benzothiazine 2,2-dioxides [5,7]. On the other hand, benzofuran derivatives occupy significant position due to their widespread occurrence in plants [8] and for their biological activities as antioxidant [9,10] and anticancer [11,12]. Based on the above observations, our goal deals with study of the reaction of CSI with 4-methoxy-5-acetyl-6-hydroxybenzofuran (visnaginone) (1a), 4,7-dimethoxy-5-acetyl-6-hydroxybenzofuran (khellinine) (1b), and their α,β-unsaturated keto derivatives 6a–g and 7a–g to obtain novel furo[2,3-g]-1,2,3-benzothiazine-7,7-dioxide derivatives and evaluating their radical-scavenging and anticancer activities.

Experimental

Synthesis

Melting points were determined in open capillary tubes on an Electrothermal 9100 digital melting point apparatus (Mount Holly, New Jersey, USA) and were uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer (USA) and were found within ±0.4% of the theoretical values. IR spectra were recorded on a Perkin-Elmer 1600 FTIR (USA) and were uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer (USA) and were found within ±0.4% of the theoretical values.

Synthesis of compounds 2a and 2b

To a stirred solution of compound 1a or 1b (5 mmol) in dry benzene (10 mL), was added a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol) in dry benzene (5 mL) at 0–5 °C during 20 min and the stirring was continued for additional 1 h at the same temperature and then for 30 min at room temperature. The reaction mixture was set aside at refrigerator temperature. The solvent was evaporated in vacuo, the residue was triturated with acetone–water (1:10, 11 mL) and allowed to stay for 1 h. The solution was neutralized by addition of 5% sodium hydrogen carbonate and the separated solid was filtered off, washed with water, air-dried, and crystallized from absolute ethanol to give 3a (33%) or 3b (35%), respectively.

Method B

To a stirred solution of compound 1a or 1b (10 mmol) in dry toluene (40 mL), a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol) in dry toluene (5 mL) was added within 15 min. The reaction mixture was heated at 100–105 °C for 3 h. Toluene was evaporated under vacuo, and the residue was treated with cold water (50 mL). The solid that formed was filtered off, washed with water, air-dried, and crystallized from absolute ethanol to give 3a (45%) or 3b (52%), respectively.
ture. The reaction mixture was set aside at refrigerator overnight. The solid that formed was filtered off, air-dried, and crystallized from ethanol–water (10:1).

1-(4-Methoxy-6-(N-chlorosulfonyl carbamatonbenzofuran-5-yl) ethanone 4a

R = H; m.p. 100–2 °C; yield 65%. – IR (KBr): ν = 3128 (NH), 1730, 1645 (C==O), 1600 (C=C), 1375, 1157 (SO₂), 1110, 1066, 1009 (C=O–C), 740 cm⁻¹ (Cl). – 1H NMR (DMSO-d₆): δ = 2.91 (s, 3H, CH₃), 3.99 (s, 3H, OCH₃), 7.21 (s, 1H, H-7), 7.99 (s, 1H, H-3), 8.21 (d, 1H, H-2), 9.11 (s, 1H, NH). - C₁₂H₁₂ClN₂O₅S (346.73): calcd. C 41.41; H 2.69; N 3.99.

A mixture of compounds 4a or 4b (10 mmol) and piperidine (0.85 mL, 10 mmol) in dry dichloromethane (20 mL) containing triethylamine (0.5 mL) was stirred at room temperature for 5 h for compound 4a, 7 h for compound 4b, and then left overnight at room temperature. The reaction mixture was poured onto water and the precipitate that formed was filtered off, air-dried, and crystallized from methanol.

N-Piperidinosulfonyl-(4-methoxy-5-acetylbenzofuran-6-yl) carbamate 5a

R = H; m.p. 220–2 °C; yield 35%. – IR (KBr): ν = 3128 (NH), 1725, 1678 (C==O), 1575 (C=C), 1370, 1152 (SO₂), 1101, 1099, 1009 cm⁻¹ (C=O–C). – 1H NMR (DMSO-d₆): δ: 2.67 (s, 3H, CH₃), 2.99–3.22 (m, 10H, piperidinyl), 3.91 (s, 3H, OCH₃), 6.66 (s, 1H, H-7), 7.01 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.71 (s, 1H, NH). - C₁₇H₁₂N₂O₅S (396.41): calcd. C 41.51; H 5.09; N 7.07; found C 41.32; H 5.01; N 6.69.

N-Piperidinosulfonyl-(4,7-dimethoxy-5-acetylbenzofuran-6-yl) carbamate 5b

R = OCH₃; m.p. 122–4 °C; yield 55%. – IR (KBr): ν = 3132 (NH), 1732, 1686 (C==O), 1578 (C=C), 1372, 1153 (SO₂), 1111, 1099, 1066, 1001 cm⁻¹ (C=O–C). – 1H NMR (DMSO-d₆): δ: 2.69 (s, 3H, CH₃), 3.91, 4.21 (2s, 6H, 2OCH₃), 7.01 (d, 1H, H-3), 8.22 (d, 1H, H-2), 8.91 (s, 1H, NH). - C₁₇H₁₂ClN₂O₅S (377.75): calcd. C 41.41; H 2.60; N 3.91; found C 41.11; H 3.22; N 3.56.

Synthesis of compounds 8a and 9a

To a stirred solution of compound 6a or 7a (5 mmol) in dry benzene (10 mL), was added a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol) in dry benzene (5 mL) at 0–5 °C during 20 min and the stirring was continued for additional 1 h at the same temperature and then for 30 min at room temperature. The reaction mixture was set aside at refrigerator overnight. The solid that formed was filtered off, air-dried, and crystallized from benzene.

N-(4,7-Dimethoxy-6-(N-chlorosulfonyl carbamatonbenzofuran-5-yl)-3-phenylprop-2-ene)chlorosulfonyl amine 8a

R = H; Ar=C₆H₅; m.p. 120–2 °C; yield 65%. – IR (KBr): ν = 3182 (NH), 1678 (C=O), 1620 (C=N), 1567 (C=C), 1375, 1152 (SO₂), 1111, 1066, 1009 (C=O–C), 750 cm⁻¹ (Cl). – 1H NMR (DMSO-d₆): δ = 3.99 (s, 3H, OCH₃), 6.66 (s, 1H, H-7), 7.11–7.31 (m, 5H, Ar=H), 7.01, 7.66 (2d, 2H, CH–CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.57 (s, 1H, NH). - C₁₉H₁₄Cl₂N₃O₅S (533.36): calcd. C 42.79; H 2.65; N 5.25; found C 42.55; H 2.88; N 5.35.

Synthesis of compounds 10a and 11a

Method A: cyclization of 8a and 9a

To a cold stirred solution of compound 8a or 9a (10 mmol) in dry dichloromethane (10 mL), a solution of triethylamine (0.5 mL) in dry dichloromethane (2.5 mL) was added dropwise during 5 min. The reaction mixture was stirred for 5 h at room temperature. The solvent was evaporated under vacuo, and the residue was triturated with acetone–water (1:10, 11 mL) and allowed to stay for 1 h. The solution was neutralized by addition of 5% sodium hydrogen carbonate, and the separated solid was filtered off, washed with water, air-dried, and crystallized from absolute ethanol to give 10a (30%) or 11a (32%), respectively.

Method B

To a stirred solution of compound 6a or 7a (10 mmol) in dry toluene (40 mL), a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol) in dry toluene (5 mL) was added within 15 min. The reaction mixture was heated at 100–105 °C for 3 h. Toluene was evaporated under vacuo and the residue was triturated with cold water (50 mL). The solid that formed was filtered off, washed with water, air-dried, and crystallized from absolute ethanol to give 10a (77%) or 11a (78%), respectively.

4-Methoxy-5-styrylfuro[3,2-g]1,2,3-benzoxathiazine-7,7-dioxide 10a

R = H; Ar=C₆H₅; m.p. 92–4 °C. – IR (KBr): ν = 1620 (C=N), 1569 (C=C), 1375,1145 (SO₂), 1120, 1119, 1110 cm⁻¹ (C=O–C). – 1H NMR (DMSO-d₆): δ = 4.32 (s, 3H, OCH₃), 6.88 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH–CH), 7.32–7.68 (5H, Ar=H), 7.90 (d, 1H, H-3), 8.12 (d, 1H, H-2). – 13C NMR (DMSO-d₆): δ = 61.2 (OCH₃), 96.3–155.2 (Ar=CH). – EI-MS: m/z (%) = 355
4-Methoxy-5-(4-chlorostyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide 10b

R = H; Ar= C6H4Cl-; m.p. 125-7 °C; yield 52 %. – IR (KBr): \( \nu = 1620 (C=O), 1599, 1375, 1155 (SO\textsubscript{2}), 1120, 1119, 1110 \text{ cm}^{-1} (C=O). – ^{1}H NMR (DMSO-\textsubscript{d6}) \delta: 4.32 (2s, 3H, OCH\textsubscript{3}), 6.77 (s, 1H, H-9), 7.00, 7.12 (2d, 2H, CH=CH), 7.21-7.76 (m, 4H, Ar-H), 7.99 (d, 1H, H-3), 8.12 (d, 1H, H-2). – ^{13}C NMR (DMSO-\textsubscript{d6}) \delta: 61.9 (OCH\textsubscript{3}), 96.2-131.1 (Ar-C), – EI-MS: \( m/z (\%) = 389/391\) (M\textsuperscript{+} / M\textsuperscript{+} + 2, 8/2). – C\textsubscript{18}H\textsubscript{13}NO\textsubscript{3}S\textsubscript{2}(389.81): calcd. C 55.46; H 3.10; N 5.39; found C 55.33; H 3.22; N 3.34.

4-Methoxy-5-(4-fluorostyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide 10c

R = H; Ar= C6H4F-; m.p. 93-5 °C; yield 42 %. – IR (KBr): \( \nu = 1620 (C=O), 1601 (C=O), 1375, 1145 (SO\textsubscript{2}), 1120, 1119, 1110 \text{ cm}^{-1} (C=O). – ^{1}H NMR (DMSO-\textsubscript{d6}) \delta: 4.32 (2s, 3H, OCH\textsubscript{3}), 6.77 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH=CH), 7.31-7.68 (m, 4H, Ar-H), 7.99 (d, 1H, H-3), 8.22 (d, 1H, H-2). – ^{13}C NMR (DMSO-\textsubscript{d6}) \delta: 60.2 (OCH\textsubscript{3}), 92.8-151.9 (Ar-C), – EI-MS: \( m/z (\%) = 373\) (M\textsuperscript{+}, 8). – C\textsubscript{18}H\textsubscript{13}FNO\textsubscript{3}S\textsubscript{2}(373.35): calcd. C 57.91; H 3.24; N 3.75; found C 58.05; H 3.11; N 3.30.

4-Methoxy-5-(4-methoxystyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide 10d

R = H; Ar= C6H4OCH\textsubscript{3}-; m.p. 162-4 °C; yield 90 %. – IR (KBr): \( \nu = 1620 (C=O), 1575 (C=O), 1375, 1175 (SO\textsubscript{2}), 1120, 1119, 1110 \text{ cm}^{-1} (C=O). – ^{1}H NMR (DMSO-\textsubscript{d6}) \delta: 3.99, 4.30 (2s, 6H, 2OCH\textsubscript{3}), 6.77 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH=CH), 7.35-7.89 (m, 4H, Ar-H), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-\textsubscript{d6}) \delta: 55.4, 61.2 (OCH\textsubscript{3}), 96.3-161.8 (Ar-C), 172.3 (C=O). – EI-MS: \( m/z (\%) = 385\) (M\textsuperscript{+}, 40). – C\textsubscript{18}H\textsubscript{13}NO\textsubscript{3}S\textsubscript{2}(385.39): calcd. C 59.21; H 3.92; N 3.63; found C 59.01; H 4.00; N 3.55.

4-Methoxy-5-(3,4,5-trimethoxystyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide 10e

R = H; Ar= C6H4(OCH\textsubscript{3})\textsubscript{3}-; m.p. 104-6 °C; yield 97 %. – IR (KBr): \( \nu = 1620 (C=O), 1589 (C=O), 1375, 1175 (SO\textsubscript{2}), 1120, 1119, 1110, 1008 \text{ cm}^{-1} (C=O). – ^{1}H NMR (DMSO-\textsubscript{d6}) \delta: 3.89, 4.01, 4.12, 4.34 (4s, 12H, 4OCH\textsubscript{3}), 6.77 (s, 1H, H-9), 7.01, 7.32 (2d, 2H, CH=CH), 7.55-7.89 (2d, 2H, Ar-H), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-\textsubscript{d6}) \delta: 55.9, 59.9, 60.0, 61.0 (OCH\textsubscript{3}), 96.0-159.2 (Ar-C), 172.4 (C=O). – EI-MS: \( m/z (\%) = 445\) (M\textsuperscript{+}, 43). – C\textsubscript{21}H\textsubscript{15}NO\textsubscript{3}S\textsubscript{2}(445.44): calcd. C 56.62; H 4.30; N 3.14; found C 56.44; H 4.11; N 3.30.
4.9-dimethoxy-5-[(4-methoxy styryl)[furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide 11Id

R = OCH3; Ar=C6H4OCH3-3,4-; m.p. 150 °C dec.; yield 68%. – IR (KBr): ν = 1620 (C=O), 1596 (C=C), 1385, 1135 (SO2), 1120, 1119, 1110, 1009 cm⁻¹ (C=O–C). – 1H NMR (DMSO-d6): δ: 3.88, 3.99, 4.20 (3s, 9H, 3OCH3), 7.01–7.39 (m, 4H, Ar–H), 7.66, 7.72 (2d, 2H, CH–CH), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – 13C NMR (DMSO-d6): δ: 55.4, 61.6, 61.9 (OCH3). 106.2–151.1 (Ar=C), 172.4 (C=N). – EI-MS: m/z (%): = 415 (M+1, 62%); C20H17NO7S4 (415.42); calculated C 55.57; H 4.22; N 2.77.

4.9-Dimethoxy-5-[(3,4,5-trimethoxy styryl)[furo[3,2-g]-1,2,3-benzoxathi azine-7,7-dioxide 11e

R = OCH3; Ar=C6H4OCH3-3,4,5-; m.p. 120 °C dec.; yield 72%. – IR (KBr): ν = 1621 (C=O), 1585 (C=C), 1375, 1135 (SO2), 1120, 1118, 1110, 1009 cm⁻¹ (C=O–C). – 1H NMR (DMSO-d6): δ: 3.88, 3.99, 3.99, 4.01, 4.22 (5s, 15H, 5OCH3), 7.01 (s, 2H, Ar–H), 7.52,7.82 (2d, 2H, CH–CH), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – 13C NMR (DMSO-d6): δ: 55.6, 59.9, 60.0, 61.5, 62.7 (OCH3), 103.9–153.1 (Ar=C). – EI-MS: m/z (%): = 475 (M+1, 11%); C20H17NO7S4 (475.47); calculated C 55.57; H 4.45; N 2.95; found C 55.64; H 4.22; N 2.77.

Synthesis of compounds 12ab and 13ab

To a stirred solution of the appropri ate α,β-unsaturated keto derivatives 6a, 6b, 7a or 7g (10 mmol) in dry benzene (10 mL), was added a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol in dry benzene 5 mL) at 0–5 °C during 20 min and the stirring was continued for additional 1 h at the same temperature and for 30 min at room temperature. The reaction mixture was set aside at refrigerator overnight. The solid that formed was filtered off, air-dried, and crystalized from ethanol–water (10:1).

N-Chlorosulfonyl 4-methoxy-5-[(3-phenoacyl acryloxy)]benzofuran-6-yI carbamates 12ab

R = H; Ar=C6H5; m.p. 202–4 °C; yield 70%. – IR (KBr): ν = 3180 (NH), 1720, 1645 (C=O), 1545 (C=C), 1370, 1150 (SO2), 1110, 1109, 1009 (C=O–C), 742 cm⁻¹ (Cl). – 1H NMR (DMSO-d6): δ: 3.99 (s, 3H, OCH3), 6.66 (s, 1H, H-7), 7.21–7.45 (m, 5H, phenyl), 7.92 (d, 1H, H-3), 7.69, 7.71 (2d, 2H, CH–CH), 8.12 (d, 1H, H-2), 9.71 (s, 1H, NH). – C20H19ClNO5S (435.83); calculated C 52.36; H 3.24; N 3.21; found C 52.14; H 3.07; N 3.03.

N-Chlorosulfonyl 4-methoxy-5-[(3-indo lyl acryloxy)]benzofuran-6-yI carbamates 13ab

R = H; Ar = 3-indolyl; m.p. 194–6 °C; yield 75%. – IR (KBr): ν = 3280, 3218 (NH), 1702, 1665 (C=O), 1575 (C=C), 1370, 1151 (SO2), 1111, 1109, 1009 (C=O–C), 745 cm⁻¹ (Cl). – 1H NMR (DMSO-d6): δ: 4.21 (s, 3H, OCH3), 6.66 (s, 1H, H-7), 7.21–7.45 (m, 4H, indolyl), 7.12, 7.66 (2d, 2H, CH–CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.25 (s, 1H, indolyl 2-H), 8.70 (s, 1H, NH), 9.71 (s, 1H, indolyl NH). – C21H15ClNO5S (474.87); calculated C 53.11; H 3.18; N 5.90; found C 53.27; H 3.01; N 5.72.

N-Chlorosulfonyl 4,7-dimethoxy-5-[(3-phenoacyl acryloxy)]benzofuran-6-yI carbamates 13ab

R = OCH3; Ar=C6H5; m.p. 238–40 °C; yield 70%. – IR (KBr): ν = 3180 (NH), 1730, 1670 (C=O), 1575 (C=C), 1370, 1157 (SO2), 1111, 1066, 1009, 1001 (C=O–C), 745 cm⁻¹ (Cl). – 1H NMR (DMSO-d6): δ: 3.99, 4.21 (2s, 6H, 2OCH3), 7.11–7.54 (m, 5H, phenyl), 7.66, 7.76 (2d, 2H, CH–CH), 7.91 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.75 (s, 1H, NH). – C20H15ClNO5S (465.86); calculated C 51.56; H 3.46; N 3.01; found C 51.46; H 3.31; N 3.11.

N-Chlorosulfonyl 4,7-dimethoxy-5-[(3-indolyl acryloxy)]benzofuran-6-yI carbamates 13ab

R = OCH3; Ar = 3-indolyl; m.p. 141–3 °C; yield 80%. – IR (KBr): ν = 3200, 3128 (NH), 1701, 1654 (C=O), 1570 (C=C), 1375, 1157 (SO2), 1111, 1099, 1066, 1001 (C=O–C), 745 cm⁻¹ (Cl). – 1H NMR (DMSO-d6): δ: 3.91, 4.21(2s, 6H, 2OCH3), 7.21–7.45 (m, 4H, indolyl), 7.66, 7.79 (2d, 2H, CH–CH), 7.91 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.57 (s, 1H, indolyl 2-H), 9.30 (s, 1H, NH), 10.51 (s, 1H, indolyl NH). – C22H13ClNO5S (504.90); calculated C 52.33; H 3.39; N 5.55; found C 52.21; H 3.30; N 5.42.

Synthesis of compounds 14ab and 15ab

A mixture of compounds 12ab or 13ab (10 mmol) and piperidine (0.85 mL, 10 mmol) in dry 1,4-dioxane (20 mL) containing triethylamine (0.5 mL) was stirred at room temperature for 5–7 h and then left overnight at room temperature. The reaction mixture was poured onto water and the precipitate that formed was filtered off, dried, and crystalized from methanol.
N-Piperidinosulfonyl 4-methoxy-5- (3-phenylacryloyl) benzofuran-6-yl carbamates 14a

R = H; Ar = C6H5; m. p. 78–80 °C; yield 40%. – IR (KBr): \( \nu = 3110, 1620, 1290 \text{ cm}^{-1} \). – 1H NMR (DMSO-\( d_6 \)).

N-Piperidinosulfonyl 4-methoxy-5- (3-indolyl) (acyrloyl) benzofuran-6-yl carbamates 14b

R = H; Ar = 3-indolyl; m. p. 262–4 °C; yield 42%. – IR (KBr): \( \nu = 3200, 3120, 1700, 1651 \text{ cm}^{-1} \). – 1H NMR (DMSO-\( d_6 \)).

N-Piperidinosulfonyl 4,7-dimethoxy-5- (3-phenylacryloyl) benzofuran-6-yl carbamates 15a

R = OCH3; Ar = C6H5; m. p. 147–9 °C; yield 55%. – IR (KBr): \( \nu = 1760, 1600, 1060 \text{ cm}^{-1} \). – 1H NMR (DMSO-\( d_6 \)).

N-Piperidinosulfonyl 4,7-dimethoxy-5- (3-indolyl) (acyrloyl) benzofuran-6-yl carbamates 15b

R = OCH3; Ar = 3-indolyl; m. p. 91–3 °C; yield 45%. – IR (KBr): \( \nu = 3280, 3181, 1699, 1671 \text{ cm}^{-1} \). – 1H NMR (DMSO-\( d_6 \)).

Biological assay

DPPH radical-scavenging activity

Sixteen new target synthesized compounds 3a-b, 10a-g, and 11a-g were screened for their DPPH radical-scavenging activity using the procedure of Viuda-Martos et al. [17]. A volume of 20 \( \mu \text{L} \) of methanolic solution of test compounds of 100 \( \mu \text{g/mL} \) was added to 2 \( \mu \text{L} \) of \( 6 \times 10^{-3} \text{ mol L}^{-1} \) methanolic solution of DPPH (2.3659 mg DPPH in 100 mL methanol). The mixture was shaken vigorously and allowed to stand for 1 h in a dark room. Ascorbic acid (Sigma–Aldrich Chemie GmbH, Tauferkirchen, Germany) was used as a reference. The decrease in absorbance at 517 nm was determined using microplate ELIZA reader (ASYS Hitech GmbH, Austria). Absorbance of DPPH radical without sample was used as negative control. The percentage of scavenging activity was calculated according to the formula, \( \% I = \left( \frac{A_B - A_s}{A_B} \right) 	imes 100 \), where \( I = \text{DPPH inhibition}\% \) and \( A_s = \text{absorbance of a tested sample at the end of the reaction (1 h)} \) and \( A_B = \text{absorbance of a control (t = 0 h)} \). All tests and analyses were done in triplicate and the results were averaged.

Cell culture

A-549 (human lung carcinoma), MCF7 (human breast carcinoma), and HCT-116 (human colon carcinoma) cell lines were obtained from Karolinska Institute, Stockholm, Sweden. All cells were maintained in RPMI 1640 medium, except for A-549 cancer cells which were maintained in DMEM medium (Lonza Biowadtkar, Belgium). All the media were supplemented with 1% antibiotic–antimycotic mixture (10,000 U mL\(^{-1}\) potassium penicillin, 10,000 \( \mu \text{g/mL} \) streptomycin sulfate, 25 \( \mu \text{g/mL} \) amphotericin B, and 1% L-glutamine (Biowest, USA).

MTT cytotoxicity assay

Cell viability was investigated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Bio Basic Canada Inc., Canada) assay [18]. This reaction depends on the mitochondrial reduction of yellow MTT into purple formazan. All the preceding steps were carried out in sterile laminar air flow cabinet Biosafety class II level (Baker, Sheldon, TC2323, Cornelius, OR, USA). All incubations were done at 37 °C in 5% CO\(_2\) incubator in humidified atmosphere (Sheldon, TC2323, Cornelius, OR, USA). Cells were seeded into 96-well microtiter plastic plates at the concentration of \( 10^4 \) cells per well and allowed to adhere for 24 h. Medium was aspirated and fresh medium (without serum) was added to the cells with various concentrations of the test compounds (100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 \( \mu \text{g/mL} \) in DMSO) and incubated for 48 h. Medium was aspirated and 40 \( \mu \text{L} \) MTT salt (2.5 \( \mu \text{g/mL} \) ) was added to each well and incubated for further 4 h. To stop the reaction and dissolve any formed formazan crystals, 200 \( \mu \text{L} \) of 10% sodium dodecyl sulfate (SDS) was added to each well and incubated overnight at 37 °C. The amount of formazan product was measured at 595 nm with a reference wavelength of 620 nm as a background using a microplate reader (Bio-Rad Laboratories, model 3350, USA). For the untreated cells (negative control), medium was added instead of the test compounds. A positive control Adriamycin® (doxorubicin) (Mr = 579.9) was used as a known cytotoxic natural agent giving 100% inhibition. Dimethyl sulfoxide (DMSO) was the vehicle used for dissolution of tested compound, and its final concentration on the cells was less than 0.2%.

IC\(_{50}\) was calculated for the samples and negative control (cells with vehicle) by the probit analysis using simple \( t\)-test (SPSS statistical analysis software package/version 11.0, SPSS Inc., (IL), Chicago, USA).
**Results and discussion**

**Chemistry**

The synthetic routes of the titled compounds are outlined in Schemes 1 and 2. Addition of two equivalent of chlorosulfonyl isocyanate (CSI) to 4-methoxy-5-acetyl-6-hydroxybenzofuran (1a) and 4,7-dimethoxy-5-acetyl-6-hydroxybenzofuran (1b) in dry benzene at 0–5 °C led to the formation of N-(4-methoxy-6-(N-chlorosulfonyl carbamate-benzofuran-5-yl)ethylidene) chlorosulfonyl amine (2a) and N-(4,7-dimethoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl)ethylidene) chlorosulfonyl amine (2b). Treatment of compound 2a or 2b with triethylamine under stirring afforded the cyclized, 4-methoxy-5-methylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide (3a) and 4,9-dimethoxy-5-methylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide (3b) with overall yield 33 and 35%, respectively (method A). This reaction probably proceeded through the intermediate A followed by losing a molecule of (ClSO₂NCO) as reported in similar reactions [7] (Scheme 1).

On the other hand, cycloaddition reaction of CSI with compounds 1a or 1b in equimolar amounts in dry benzene (1:1 adduct) at 0–5 °C led to the formation of 1-(4-methoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl) ethanone (4a) and 1-(4,7-dimethoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl) ethanone (4b), respectively (Scheme 1). The structures of compounds 4a and 4b were confirmed upon the bases of their correct elemental analyses and spectral data (c.f. experimental section), besides the chemical evidences that; (a) compounds 4a,b showed negative ferric chloride test and positive sulfur, nitrogen, and halogen tests and (b) compounds 4a,b reacted with piperidine in presence of triethylamine and afforded the corresponding N-piperidinosulfonyl carbamates 5a and 5b (Scheme 1).
In a similar manner, $\alpha,\beta$-unsaturated keto derivative $6a$ or $7a$ reacted with two equivalent of CSI in dry benzene at 0–5°C and gave N-(4-methoxy (8a) and (4,7-dimethoxy)-6-$(N$-chlorosulfonylcarbamato-benzofuran-5-yl)-3-phenylprop-2-ene)chlorosulfonyl amines ($9a$) with yields of 52% and 60%, respectively. Cyclization of the latter compounds via their reaction with triethylamine led to the formation of 4-methoxy ($10a$) and 4,9-dimethoxy-5-styrylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ($11a$) with overall yields 30 and 32%, respectively (Scheme 2).

Due to the low yield of compounds $10a$ and $11a$ via the above two step reactions, we used the direct reaction of CSI with compounds $6a$ or $7a$ in dry toluene at 100–105°C (1:1 adduct) to give $10a$ and $11a$ with good yields 77% and 78%, respectively (Scheme 2).

The structure of all the new compounds was confirmed based on their correct elemental analyses and spectral data (c.f. experimental section).

**Biological activity**

**DPPH radical-scavenging activity**

Compounds $3a,b$, $10a$–$g$, and $11a$–$g$ were screened for their DPPH radical-scavenging activity using ascorbic acid as a reference. Antioxidant reacts with DPPH, which is stable free radical and converts it to 1,1-diphenyl-2-picrylhydrazine. The
The degree of discoloration indicates the scavenging potential of the antioxidant compounds. From the data obtained, only compounds 10a, 11c, 11e, and 11g showed moderate DPPH radical-scavenging activity of 59.7%, 50.3%, 50.0%, and 53.8%, respectively, than the rest of the screened compounds, which showed slightly activity compared to ascorbic acid of 96.0% at 100 μg/mL, Table 1.

### Table 1 DPPH radical-scavenging assay of the synthesized compounds.

| Compd. | Scavenging activity (%)a at 100 μg/mL |
|--------|-------------------------------------|
| 3a     | 10.6                                |
| 3b     | 30.4                                |
| 10a    | 59.7                                |
| 10b    | 12.7                                |
| 10c    | 11.1                                |
| 10d    | 7.5                                 |
| 10e    | 11.8                                |
| 10f    | 0.0                                 |
| 10g    | 20.4                                |
| 11a    | 28.5                                |
| 11b    | 17.4                                |
| 11c    | 50.3                                |
| 11d    | 17.3                                |
| 11e    | 50.0                                |
| 11f    | 9.7                                 |
| 11g    | 53.8                                |
| Negative control | –                           |
| Ascorbic acid | 96.0                           |

a Results are the mean of three independent experiments.

### Table 2 Antiproliferative activity of the newly synthesized compounds against human carcinoma cell lines.

| Compd. | Inhibition growth (%) |
|--------|-----------------------|
|        | A549 | MCF7 | HCT-116 |
| 3a     | 15.8 | 28.0 | 45.7    |
| 3b     | 4.2  | 36.0 | 0       |
| 10a    | 73.3 | 91.2 | 100     |
| 10b    | 82.7 | 99.2 | 100     |
| 10c    | 8.2  | 95.4 | 69.9    |
| 10d    | 4.9  | 85.4 | 74.8    |
| 10e    | 68.7 | 85.4 | 81.6    |
| 10f    | 21.4 | 27.2 | 4.3     |
| 10g    | 29.6 | 16.7 | 0       |
| 11a    | 81.0 | 84.4 | 100     |
| 11b    | 82.7 | 97.5 | 100     |
| 11c    | 73.8 | 92.2 | 100     |
| 11d    | 1.5  | 6.1  | 16.7    |
| 11e    | 55.2 | 80.2 | 88.0    |
| 11f    | 0    | 20.7 | 0       |
| 11g    | 0    | 0    | 1.4     |
| Negative b control | –       | – | –   |
| Doxorubicin | 100.0  | 100.0  | 100.0  |

a Concentration of test compounds and positive control (doxorubicin) 100 μg/mL.

b Untreated cells in DMSO and its final concentration on the cells was less than 0.2%.

### Table 3 IC50 of the highly antiproliferative active compounds against human cancer cell lines.

| Compd. | IC50 (μmol/mL) |
|--------|----------------|
|        | A549 | MCF7 | HCT-116 |
| 10a    | >0.78| >0.78| >0.78   |
| 10b    | >0.78| >0.78| >0.78   |
| 11a    | 0.05 | >0.78| 0.08    |
| 11b    | 0.03 | >0.78| 0.05    |
| 11c    | 0.02 | >0.78| 0.03    |
| 11e    | –   | >0.78| >0.78   |
| Doxorubicin | 0.04 | 0.07 | 0.06 |

IC50 – Concentration required inhibiting cell viability by 50%.

**Antiproliferative activity**

Compounds 3a,b, 10a–g, and 11a–g were preliminary screened for their in vitro antiproliferative activity against human lung carcinoma (A-549), human breast cancer (MCF7), and human colon cancer (HCT-116) cell lines at a concentration of 100 μg/mL, Table 2. Compounds 10a, 10b, 11a, 11b, and 11c were found to be the most active compounds with antiproliferative activity of 100% against HCT-116 cancer cell line, whereas the most active compounds against MCF7 cancer cell line was in the descending order of 10b > 11b > 10c > 11c > 10a > 10d and 10e with antiproliferative activity of 99.2%, 97.5%, 95.4%, 92.2%, 91.2%, 85.4% and 85.4%, respectively. On the other hand, compounds 10a, 10b, 11a, 11b, and 11c were found to be the most active one with antiproliferative activity of 73.3%, 82.7%, 81.0%, 82.7%, and 73.8%, respectively, against A-549 cancer cell line.

The compounds that showed antiproliferative activity higher than 70% at concentration of 100 μg/mL were used to calculate their IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. Doxorubicin was used as a reference drug, Table 3. From the data obtained, compound 11a showed potent inhibition of IC50 = 0.05 and 0.08 μmol/mL against A-549 and HCT-116, respectively, nearly as active as doxorubicin of IC50 = 0.04 and 0.06 μmol/mL, respectively.

On the other hand, compounds 11b and 11c showed higher activity with inhibition of IC50 = 0.03 and 0.02 μmol/mL, respectively, against A-549 compared to doxorubicin (IC50 = 0.04 μmol/mL). Also, compounds 11b and 11c showed higher activity with inhibition of IC50 = 0.05 and 0.03 μmol/mL, respectively, against HCT-116 compared to doxorubicin (IC50 = 0.06 μmol/mL).

From the data obtained, it is clear that compounds 11a, 11b, and 11c found to be the most active compounds against A-549 and HCT-116 cancer cell lines and their activity may be due to the presence of the methoxy donating group at the position-9 of furobenzoxathiazine. Besides that, the presence of withdrawing chlorine atom in position-9 of furo[2,3-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives 3a,b.

**Conclusions**

A novel series of 4-methoxy, and 4,9-dimethoxy-5-substituted furo[2,3-g]-1,2,3-benzoxathiazine derivatives 3a,b.
10a–g and 11a–g were prepared via reaction of 4-methoxy (1a) and 4,7-dimethoxy-5-acetyl-6-hydroxy benzofurans (1b) and their α,β-unsaturated keto derivatives 6a–g and 7a–g with chlorosulfonyl isocyanate (CSI). Compounds 10a, 11c, 11e, and 11g showed moderate DPPH radical-scavenging activity compared to ascorbic acid at 100 μg/mL. 4,9-Dimethoxy-5-substituted styrylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxides 11a, 11b, and 11c were found to be highly active against A-549 and HCT-116 cancer cell lines with IC₅₀ values ranging from 0.02 to 0.08 μmol/mL compared to doxorubicin of IC₅₀ = 0.04 and 0.06 μmol/mL, respectively.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

The authors are grateful to Micro-analytical Unit, National Research Centre, Cairo, Egypt, for carrying out elemental analyses and IR spectra. Also, the authors thank Kamel H. Shaker and NMR/Biosynthesis Department, Max Plank Institute for Chemical Ecology 07745, Jena, Germany, for carrying out NMR and mass spectra.

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