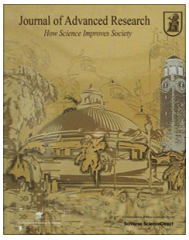
Journal of Advanced Research (2014) 5, 337–346

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

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

Eslam R. El-Sawy [a], Manal S. Ebaid [a], Heba M. Abo-Salem [a,*], Salwa El-Hallouty [b], Emad M. Kassem [c], Adel H. Mandour [a]

[a] *Chemistry Department of Natural Compounds, National Research Centre, 12311 Dokki, Giza, Egypt*
[b] *Pharmacognosy Department, National Research Centre, 12311 Dokki, Giza, Egypt*
[c] *Therapeutical Chemistry Department, National Research Centre, 12311 Dokki, Giza, Egypt*

ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 13 May 2013
Accepted 13 May 2013
Available online 21 May 2013

Keywords:
Chlorosulfonyl isocyanate (CSI)
Benzofuran
Benzoxathiazin-7,7-dioxide
DPPH radical-scavenging activity
Anticancer activity

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 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 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.

Introduction

Chlorosulfonyl isocyanate (CSI) is a remarkable reagent of exceptional reactivity [1,2]. This reagent as a uniparticulate 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

* Corresponding author. Tel.: +20 1112038775; fax: +20 33370931.
E-mail address: hb_abosalem@yahoo.com (H.M. Abo-Salem).

Peer review under responsibility of Cairo University.

Production and hosting by Elsevier

2090-1232 © 2013 Production and hosting by Elsevier B.V. on behalf of Cairo University.
http://dx.doi.org/10.1016/j.jare.2013.05.004

N-chlorosulfonyl carbamate derivatives, whereas cycloaddition of CSI with the above moieties in dry toluene at 100 °C produces 1,2,3-benzoxathiazine 2,2-dioxides [5,7]. On the other hand, benzofuran derivatives occupy significance 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 (khellinone) **(1b),** and their α,β-unsaturated keto derivatives **6a–g** and **7a–g** to obtain novel furo[2,3-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives and evaluating their DPPH 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) in KBr disks. The NMR spectra were measured with a Bruker Avance digital spectrometer (Germany) (500 and 125 M*Hz*) in DMSO-d_6 and chemical shifts was recorded in δ ppm relative to TMS as internal standard. Mass spectra (EI) were run at 70 eV with a JEOL-JMS-AX500 mass spectrometer (Japan). 4-methoxy-5-acetyl-6-hydroxy benzofuran (visnaginone) **(1a)** [13], 4,7-dimethoxy-5-acetyl-6-hydroxy benzofuran (khellinone) **(1b)** [14], α,β-unsaturated keto derivatives **6a–g** and **7a–g** [10,15,16] were prepared as reported.

*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 overnight. The solid that formed was filtered off, air-dried, and crystallized from benzene.

*N-(4-Methoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl) ethylidene)chlorosulfonyl amine **2a***

R = H; m.p. 112–4 °C; yield 44%. – IR (KBr): υ = 3200 (NH), 1645 (C═O), 1620 (C═N), 1585 (C═C), 1371, 1136 (SO_2), 1121, 1119, 1110 (C─O─C), 740 cm^{-1} (Cl). – ^{1}H NMR (DMSO-d_6) δ: 1.61 (s, 3H, CH_3), 4.20 (s, 3H, OCH_3), 6.77 (s, 1H, H-7), 7.52 (d, 1H, H-3), 8.02 (s, 1H, NH), 8.21 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 20.2 (CH_3), 60.9 (OCH_3), 92.8–159.0 (Ar─C), 166.6 (C═N). – $C_{12}H_{10}Cl_2N_2O_8S_2$ (445.25): calcd. C 32.37; H 2.26; N 6.29; found C 32.15; H 2.11; N 6.47.

*N-(4,7-Dimethoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl) ethylidine)chlorosulfonyl amine **2b***

R = OCH_3; m.p 107–9 °C; yield 32%. – IR (KBr): υ = 3218 (NH), 1665 (C═O), 1620 (C═N), 1575 (C═C), 1372, 1136 (SO_2), 1121, 1120, 1119, 1110 (C─O─C), 745 cm^{-1} (Cl). – ^{1}H NMR (DMSO-d_6) δ: 1.99 (s, 3H, CH_3), 4.01, 4.22 (2s, 6H, 2OCH_3), 7.81 (d, 1H, H-3), 8.01 (s, 1H, NH), 8.23 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 21.8 (CH_3), 61.0, 61.8 (OCH_3), 105.6–151.3 (Ar─C), 170.0 (C═N). – $C_{13}H_{12}Cl_2N_2O_9S_2$(475.28): calcd. C 32.85; H 2.54; N 5.89; found C 32.61; H 2.30; N 5.64.

*Synthesis of compounds **3a** and **3b***

*Method A: cyclization of **2a** and **2b***

To a cold stirred solution of compounds **2a** or **2b** (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 in 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 **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.

*4-Methoxy-5-methylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide **3a***. R = H; m.p. 161–3 °C. – IR (KBr): υ = 1620 (C═N), 1579 (C═C), 1375, 1165 (SO_2), 1120, 1119, 1110 cm $^{-1}$ (C─O─C). – ^{1}H NMR (DMSO-d_6) δ: 2.89 (s, 3H, CH_3), 4.40 (s, 3H, OCH_3), 6.77 (s, 1H, H-9), 7.62 (d, 1H, H-3), 8.22 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 18.5 (CH_3), 60.9 (OCH_3), 92.8–159.0 (Ar─C), 166.6 (C═N). – EI-MS: *m/z* (%) = 267 (M^+, 100). – $C_{11}H_9NO_5S$(267.26): calcd. C 49.43; H 3.39; N 5.24; found C 49.22; H 3.11; N 5.02.

*4,9-Dimethoxy-5-methylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide **3b***. R = OCH_3; m.p. 120–2 °C. – IR (KBr): υ = 1618 (C═N), 1575 (C═C), 1365, 1135 (SO_2), 1120, 1119, 1110, 1009 cm^{-1} (C─O─C). – ^{1}H NMR (DMSO-d_6) δ: 2.81 (s, 3H, CH_3), 4.11, 4.32 (2s, 6H, 2OCH_3), 7.61 (d, 1H, H-3), 8.22 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 28.8 (CH_3), 61.0 (OCH_3), 61.8 (OCH_3), 105.6-151.3 (Ar─C), 170.0 (C═N). – EI-MS: *m/z* (%) = 297 (M^+, 100). – $C_{12}H_{11}NO_6S$(297.28): calcd. C 48.48; H 3.73; N 4.71; found C 48.23; H 3.69; N 4.55.

*Synthesis of compounds **4a** and 4b*

To a stirred solution of compound **1a** or **1b** (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 tempera-

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 carbamatobenzofuran-5-yl) ethanone ***4a***

R = H; m.p. 100–2 °C; yield 65%. – IR (KBr): υ = 3128 (NH), 1730, 1645 (C═O), 1600 (C═C), 1375, 1157 (SO_2), 1110, 1066, 1009 (C─O─C), 740 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 2.91 (s, 3H, CH_3), 3.99 (s, 3H, OCH_3), 7.21 (s, 1H, H-7), 7.99 (s, 1H, H-3), 8.21 (d, 1H, H-2), 9.11 (s, 1H, NH).-$C_{12}H_{10}ClNO_7S$(346.73): calcd. C 41.45; H 2.90; N 4.03; found C 41.22; H 3.01; N 3.99.

1-(4,7-Dimethoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl) ethanone ***4b***

R = OCH_3; m.p. 122–4 °C; yield 55%. – IR (KBr): υ = 3200 (NH), 1732, 1686 (C═O), 1578 (C═C), 1372, 1153 (SO_2), 1111, 1099, 1066, 1001 cm^{-1} (C─O─C). – ^1H NMR (DMSO-d_6) δ: 2.69 (s, 3H, CH_3), 3.91, 4.21 (2s, 6H, $2OCH_3$), 7.01 (d, 1H, H-3), 8.22 (d, 1H, H-2), 8.91 (s, 1H, NH). – $C_{13}H_{12}ClNO_8S$(377.75): calcd. C 41.33; H 3.20; N 3.71; found C 41.11; H 3.22; N 3.56.

Synthesis of compounds ***5a*** *and* ***5b***

A mixture of compounds **4a** or **4b** (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 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, 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): υ = 3128 (NH), 1725, 1678 (C═O), 1575 (C═C), 1370, 1152 (SO_2), 1101, 1099, 1009 cm^{-1} (C─O─C). – ^1H NMR (DMSO-d_6) δ: 2.67 (s, 3H, CH_3), 2.99–3.22 (m, 10H, piperidinyl), 3.91 (s, 3H, OCH_3), 6.66 (s, 1H, H-7), 7.01 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.71 (s, 1H, NH). – $C_{17}H_{20}N_2O_7S$(396.41): calcd. C 51.51; H 5.09; N 7.07; found C 51.32; H 5.21; N 6.99.

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

R = OCH_3; m.p. 166–9 °C; yield 42%. – IR (KBr): υ = 3161 (NH), 1720, 1645 (C═O), 1601 (C═C), 1370, 1152 (SO_2), 1112, 1099, 1066, 1009 cm^{-1} (C─O─C). – ^1H NMR (DMSO-d_6) δ: 2.61 (s, 3H, CH_3), 2.81–3.31 (m, 10H, piperidinyl), 3.91, 4.21 (2s, 6H, $2OCH_3$), 7.61 (d, 1H, H-3), 8.12 (d, 1H, H-2), 9.51 (s, 1H, NH). – $C_{18}H_{22}N_2O_8S$(426.44): calcd. C 50.70; H 5.20; N 6.57; found C 50.55; H 5.42; N 6.36.

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-Methoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl)-3-phenyl-prop-2-ene]chlorosulfonyl amine ***8a***

R = H; Ar═C_6H_5; m.p. 120–2 °C; yield 65%. – IR (KBr): υ = 3182 (NH), 1678 (C═O), 1620 (C═N), 1567 (C═C), 1375, 1152 (SO_2), 1111, 1066, 1009 (C─O─C), 750 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 3.99 (s, 3H, OCH_3), 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). – $C_{19}H_{14}Cl_2N_2O_8S_2$(533.36): calcd. C 42.79; H 2.65; N 5.25; found C 42.55; H 2.88; N 5.35.

N-[4,7-Dimethoxy-6-(N-chlorosulfonyl carbamatobenzofuran-5-yl)-3-phenylprop-2-ene]chlorosulfonyl amine ***9a***

R = OCH_3; Ar═C_6H_5; m.p. 100–2 °C; yield 60%. – IR (KBr): υ = 3200 (NH), 1645 (C═O), 1618 (C═N), 1575 (C═C), 1370, 1150 (SO_2), 1110, 1066, 1009, 1001 (C─O─C), 748 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 3.99, 4.22 (2s, 6H, $2OCH_3$), 7.01–7.30 (m, 5H, phenyl), 7.66, 7.56 (2d, 2H, CH═CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 9.55 (s, 1H, NH). – $C_{20}H_{16}Cl_2N_2O_9S_2$(563.39): calcd. C 42.64; H 2.86; N 4.97; found C 42.44; H 3.01; N 5.10.

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═C_6H_5; m.p. 92–4 °C. – IR (KBr): υ = 1620 (C═N), 1569 (C═C), 1375,1145 (SO_2), 1120, 1119, 1110 cm^{-1} (C─O─C). – ^1H NMR (DMSO-d_6) δ: 4.32 (s, 3H, OCH_3), 6.88 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH═CH), 7.32–7.68 (m, 5H, Ar─H), 7.90 (d, 1H, H-3), 8.12 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 61.2 (OCH_3), 96.3–155.2 (Ar─C), 159.3 (C═N). – EI-MS: m/z (%) = 355

(M^+, 53). – $C_{18}H_{13}NO_5S$(355.36): calcd. C 60.84; H 3.69; N 3.94; found C 60.66; H 3.54; N 3.77.

4,9-Dimethoxy-5-styrylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11a***. R = OCH_3; Ar=C_6H_5; m.p. 95–7 °C. – IR (KBr): υ = 1620 (C=N), 1598 (C=C), 1375, 1135 (SO_2), 1120, 1119, 1110, 1109 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 4.01, 4.22 (2s, 6H, $2OCH_3$), 7.01–733 (m, 5H, Ar–H), 7.51, 7.61 (2d, 2H, CH=CH), 7.89 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 61.6, 61.9 ($2OCH_3$), 105.0–151.3 (Ar–C), 172.0 (C=N).-EI-MS: *m/z* (%) = 385 (M^+, 75). – $C_{19}H_{15}NO_6S$(385.39): calcd. C 59.21; H 3.92; N 3.63; found C 59.44; H 3.64; N 3.51.

Synthesis of compounds ***10b–g*** *and* ***11a–g***

To a stirred solution of the appropriate α,β-unsaturated keto derivatives **6b–g** or **7b–g** (10 mmol) in dry toluene (40 mL), a solution of chlorosulfonyl isocyanate (0.87 mL, 10 mmol in dry toluene 5 mL) was added during 15 min. The reaction mixture was heated at 100–105 °C for 3–4 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.

4-Methoxy-5-(4-chlorostyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***10b***

R = H; Ar=C_6H_4Cl-*p*; m.p. 125–7 °C; yield 52%. – IR (KBr): υ = 1620 (C=N), 1599 (C=C), 1375, 1155 (SO_2), 1120, 1119, 1110 (C–O–C), 740 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 4.32 (s, 3H, OCH_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-d_6) δ: 61.2 (OCH_3), 96.2–131.1 (Ar–C).-EI-MS: *m/z* (%) = 389/391 (M^+/$M^+ + 2$, 8/2). – $C_{18}H_{12}ClNO_5S$ (389.81): calcd. C 55.46; H 3.10; N 3.59; 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=C_6H_4F-*p*; m.p. 93–5 °C; yield 42%. – IR (KBr): υ = 1620 (C=N), 1601 (C=C), 1375, 1145 (SO_2), 1120, 1119, 1110 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 4.32 (s, 3H, OCH_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-d_6) δ: 60.2 (OCH_3), 92.8– 151.9 (Ar–C).-EI-MS: *m/z* (%) = 373 (M^+, 8). – $C_{18}H_{12}FNO_5S$(373.35): calcd. C 57.91; H 3.24; N 3.75; found C 58.05; H 3.11; N 3.50.

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

R = H; Ar=$C_6H_4OCH_3$-*p*; m.p. 162–4 °C; yield 90%. – IR (KBr): υ = 1620 (C=N), 1575 (C=C), 1375, 1175 (SO_2), 1120, 1119, 1110, 1009 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 3.99, 4.30 (2s, 6H, $2OCH_3$), 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-d_6) δ: 55.4, 61.2 (OCH_3), 96.3–161.8 (Ar–C), 172.3 (C=N). – EI-MS: *m/z* (%) = 385 (M^+, 40). – $C_{19}H_{15}NO_6S$(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=$C_6H_2(OCH_3)_3$-*3,4,5*; m.p. 104–6 °C; yield 97%. – IR (KBr): υ = 1620 (C=N), 1589 (C=C), 1375, 1175 (SO_2), 1120, 1119, 1110, 1109, 1008 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 3.89, 4.01, 4.12, 4.34 (4s, 12H, $4OCH_3$), 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-d_6) δ: 55.9, 59.9, 60.0, 61.0 (OCH_3), 96.0–159.2 (Ar–C), 172.4 (C=N). – EI-MS: *m/z* (%) = 445 (M^+, 43).-$C_{21}H_{19}NO_8S$ (445.44): calcd. C 56.62; H 4.30; N 3.14; found C 56.44; H 4.11; N 3.30.

4-Methoxy-5-(4-N,N-dimethylstyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***10f***

R = H; Ar=$C_6H_4N(CH_3)_2$-*p*; m.p. 92–4 °C; yield 98%. – IR (KBr): υ = 1618 (C=N), 1599 (C=C), 1375, 1135 (SO_2), 1120, 1119, 1110 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 3.01, 3.12 (2s, 6H, $2CH_3$), 4.02 (s, 3H, OCH_3), 6.77 (s, 1H, H-9), 6.99, 7.12 (2d, 2H, CH=CH), 7.24–7.57 (m, 4H, Ar–H), 7.89 (d, 1H, H-3), 8.09 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 60.0 (CH_3), 61.1 (OCH_3), 92.7–157.2 (Ar–C), 170.0 (C=N). – EI-MS: *m/z* (%) = 398 (M^+, 10). – $C_{20}H_{18}N_2O_5S$(398.43): calcd. C 60.29; H 4.55; N 7.03; found C 60.11; H 4.35; N 7.21.

4-Methoxy-5-(2-(3-indolyl)vinyl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***10g***

R = H; Ar = 3-indolyl; m.p. 134–6 °C; yield 97%. – IR (KBr): υ = 3350 (NH), 1620 (C=N), 1589 (C=C), 1375, 1135 (SO_2), 1120, 1119, 1110 cm^{-1} (C–O–C). – ^1H NMR (DMSO-d_6) δ: 4.22 (s, 3H, OCH_3), 6.77 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH=CH), 7.24–7.57 (m, 4H, Ar–H), 7.99 (s, 1H, indolyl 2-H), 8.12 (d, 1H, H-3), 8.34 (d, 1H, H-2), 9.91 (s, 1H, NH). – ^{13}C NMR (DMSO-d_6) δ: 60.8 (OCH_3), 105.8–158.5 (Ar–C), 107.0 (C=N). – EI-MS: *m/z* (%) = 394 (M^+, 1). – $C_{20}H_{14}N_2O_5S$(394.4): calcd. C 60.91; H 3.58; N 7.10; found C 60.87; H 3.42; N 7.22.

4,9-Dimethoxy-5-(4-chlorostyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11b***

R = OCH_3; Ar=C_6H_4Cl-*p*; m.p. 113–5 °C; yield 60%. – IR (KBr): υ = 1620 (C=N), 1585 (C=C), 1375, 1135 (SO_2), 1120, 1119, 1110, 1009 (C–O–C), 740 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 4.01, 4.22 (2s, 6H, $2OCH_3$), 6.97, 7.11 (2d, 2H, CH=CH), 7.24–7.67 (m, 4H, Ar=H), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 60.6, 60.9 ($2OCH_3$), 105.0–152.8 (Ar=C), 170.0 (C=N). – EI-MS: *m/z* (%) = 419 (M^+, 34). – $C_{19}H_{14}ClNO_6S$(419.84): calcd. C 54.36; H 3.36; N 3.34; found C 54.44; H 3.11; N 3.12.

4,9-Dimethoxy-5-(4-fluorostyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11c***

R = OCH_3; Ar=C_6H_4F-*p*; m.p. 130–2 °C; yield 82%. – IR (KBr): υ = 1620 (C=N), 1596 (C=C), 1385, 1135 (SO_2), 1120, 1119, 1110, 1009 cm^{-1} (C=O=C). – ^1H NMR (DMSO-d_6) δ: 4.01, 4.22 (2s, 6H, $2OCH_3$), 7.21–7.37 (m, 4H, Ar=H), 7.51, 7.62 (2d, 2H, CH=CH), 7.99 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 60.4, 60.9 ($2OCH_3$), 105.5–152.6 (Ar=C), 164.6 (C=N). – EI-MS: *m/z*

(%) = 403 (M^+, 11). – $C_{19}H_{14}FNO_6S$(403.38): calcd. C 56.57; H 3.50; N 3.47; found C 56.44; H 3.35; N 3.24.

4,9-dimethoxy-5-(4-methoxystyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11d***

R = OCH_3; Ar=$C_6H_4OCH_3$-*p*; m.p. 150 °C dec.; yield 68%. – IR (KBr): υ = 1620 (C=N), 1596 (C=C), 1385, 1135 (SO_2), 1120, 1119, 1110, 1009 cm^{-1} (C=O=C). – ^1H NMR (DMSO-d_6) δ: 3.88, 3.99, 4.20 (3s, 9H, 3OCH_3), 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). – ^{13}C NMR (DMSO-d_6) δ: 55.4, 61.6, 61.9 (OCH_3), 106.2–151.1 (Ar=C), 172.4 (C=N). – EI-MS: *m*/*z* (%) = 415 (M^+, 62). – $C_{20}H_{17}NO_7S$(415.42): calcd. C 57.82; H 4.12; N 3.37; found C 57.65; H 4.22; N 3.11.

4,9-Dimethoxy-5-(3,4,5-trimethoxystyryl)furo[3,2-g]-1,2,3-benzoxathi-azine-7,7-dioxide ***11e***

R = OCH_3; Ar=$C_6H_2(OCH_3)_3$-*3,4,5*; m.p. 120 °C dec.; yield 72%. – IR (KBr): υ = 1621 (C=N), 1585 (C=C), 1375, 1135 (SO_2), 1120, 1118, 1110, 1109, 1009 cm^{-1} (C=O=C). – ^1H NMR (DMSO-d_6) δ: 3.88, 3.99, 3.99, 4.01, 4.22 (5s, 15H, 5OCH_3), 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). – ^{13}C NMR (DMSO-d_6) δ: 55.6, 59.9, 60.0, 61.5, 62.7 (OCH_3), 103.9–153.1 (Ar=C). – EI-MS: *m*/*z* (%) = 475 (M^+, 11). – $C_{22}H_{21}NO_9S$(475.47): calcd. C 55.57; H 4.45; N 2.95; found C 55.44; H 4.22; N 2.77.

4,9-Dimethoxy-5-(4-N,N-dimethylstyryl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11f***

R = OCH_3; Ar=$C_6H_4N(CH_3)_2$-*p*; m.p. 86 °C dec.; yield 93%. – IR (KBr): υ = 1620 (C=N), 1589 (C=C), 1375, 1135 (SO_2), 1121, 1119, 1110, 1009 cm^{-1} (C=O=C). – ^1H NMR (DMSO-d_6) δ: 2.31, 2.67 (2s, 6H, 2CH_3), 4.01, 4.22 (2s, 6H, 2OCH_3), 6.10, 6.31 (2d, 2H, CH=CH), 7.67 (d, 1H, H-3), 8.24 (d, 1H, H-2). – ^{13}C NMR (DMSO-d_6) δ: 21.0, 29.8 (CH_3), 60.5, 61.8 (OCH_3), 105.9–150.3 (Ar=C), 170.0 (C=N).-EI-MS: *m*/*z* (%) = 428 (M^+, 17). – $C_{21}H_{20}N_2O_6S$(428.46): calcd. C 58.87; H 4.70; N 6.54; found C 58.74; H 4.65; N 6.44.

4,9-Dimethoxy-5-(2-(3-indolyl)vinyl)furo[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide ***11g***

R = OCH_3; Ar = 3-indolyl; m.p. 129–31 °C; yield 86%. – IR (KBr): υ = 3332 (NH), 1618 (C=N), 1585 (C=C), 1375, 1135 (SO_2), 1121, 1119, 1110 cm^{-1} (C=O=C). – ^1H NMR (DMSO-d_6) δ: 3.99, 4.12 (2s, 6H, 2OCH_3), 6.99, 7.12 (2d, 2H, CH=CH), 7.21–7.47 (m, 4H, Ar=H), 7.9 (s, indolyl 2-H), 8.12 (d, 1H, H-3), 8.34 (d, 1H, H-2), 9.91 (s, 1H, NH). – ^{13}C NMR (DMSO-d_6) δ: 60.5, 61.8 (OCH_3), 105.9–150.3 (Ar=C), 166.3 (C=N). – EI-MS: *m*/*z* (%) = 424(M^+, 1). – $C_{21}H_{16}N_2O_6S$ (424.43): calcd. C 59.43; H 3.80; N 6.60; found C 59.22; H 3.65; N 6.45.

Synthesis of compounds ***12a,b*** *and* ***13a,b***

To a stirred solution of the appropriate α,β-unsaturated keto derivatives **6a, 6g, 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 crystallized from ethanol–water (10:1).

N-Chlorosulfonyl 4-methoxy-5-(3-phenylacryloyl)benzofuran-6-yl carbamates ***12a***

R = H; Ar=C_6H_5; m.p. 202–4 °C; yield 70%. – IR (KBr): υ = 3180 (NH), 1720, 1645 (C=O), 1545 (C=C), 1370, 1150 (SO_2), 1110, 1109, 1009 (C=O=C), 742 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 3.99 (s, 3H, OCH_3), 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). – $C_{19}H_{14}ClNO_7S$(435.83): calcd. 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-(3-indolyl)acryloyl)benzofuran-6-yl carbamates ***12b***

R = H; Ar = 3-indolyl; m.p. 194–6 °C; yield 75%. – IR (KBr): υ = 3280, 3218 (NH), 1702, 1665 (C=O), 1575 (C=C), 1370, 1151 (SO_2), 1111, 1110, 1009 (C=O=C), 745 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 4.21 (s, 3H, OCH_3), 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). – $C_{21}H_{15}ClN_2O_7S$ (474.87): calcd. 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-phenylacryloyl)benzofuran-6-yl carbamates ***13a***

R = OCH_3; Ar=C_6H_5; m.p. 238–40 °C; yield 70%. – IR (KBr): υ = 3180 (NH), 1730, 1670 (C=O), 1575 (C=C), 1370, 1157 (SO_2), 1111, 1066, 1009, 1001 (C=O=C), 745 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 3.99, 4.21 (2s, 6H, 2OCH_3), 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). – $C_{20}H_{16}ClNO_8S$(465.86): calcd. 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-(3-indolyl)acryloyl) benzofuran-6-yl carbamates ***13b***

R = OCH_3; Ar = 3-indolyl; m.p. 141–3 °C; yield 80%. – IR (KBr): υ = 3200, 3128 (NH), 1701, 1654 (C=O), 1570 (C=C), 1375, 1157 (SO_2), 1111, 1099, 1066, 1001 (C=O=C), 745 cm^{-1} (Cl). – ^1H NMR (DMSO-d_6) δ: 3.91, 4.21(2s, 6H, 2OCH_3), 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), 9.50 (s, 1H, NH), 10.51 (s, 1H, indolyl NH). – $C_{22}H_{17}ClN_2O_8S$(504.90): calcd. C 52.33; H 3.39; N 5.55; found C 52.21; H 3.30; N 5.42.

Synthesis of compounds ***14a,b*** *and* ***15a,b***

A mixture of compounds **12a,b** or **13a,b** (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 crystallized from methanol.

N-Piperidinosulfonyl 4-methoxy-5-(3-phenylacryloyl) benzofuran-6-yl carbamates ***14a***

R = H; Ar=C_6H_5; m.p. 78–80 °C; yield 40%. – IR (KBr): υ = 3180 (NH), 1703, 1671 (C=O), 1575 (C=C), 1370, 1152 (SO_2), 1009, 1000 cm^{-1} (C=O=C). – 1H NMR (DMSO-d_6) δ: 2.66–3.21 (m, 10H, piperidinyl), 3.91 (s, 3H, OCH_3), 6.67 (s, 1H, H-7), 7.01–7.22 (m, 5H, phenyl), 7.71, 7.79 (2d, 2H, CH=CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 9.71 (s, 1H, NH). – $C_{24}H_{24}N_2O_7S$(484.52): calcd. C 59.49; H 4.99; N 5.78; found C 59.32; H 4.85; N 5.64.

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

R = H; Ar = 3-indolyl; m.p. 262–4 °C; yield 42%. – IR (KBr): υ = 3200, 3120 (NH), 1700, 1651 (C=O), 1545 (C=C), 1730, 1152 (SO_2), 1111, 1009, 1000 cm^{-1} (C=O=C). – ^{1}H NMR (DMSO-d_6) δ: 2.61–3.22 (m, 10H, piperidinyl), 3.91 (s, 3H, OCH_3), 6.61 (s, 1H, H-7), 7.11–7.45 (m, 4H, indolyl), 7.71, 7.79 (2d, 2H, CH=CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.25 (s, 1H, indolyl 2-H), 8.71 (s, 1H, NH), 9.71 (s, 1H, indolyl NH). – $C_{26}H_{25}N_3O_7S$(523.56): calcd. C 59.65; H 4.81; N 8.03; found C 59.51; H 4.67; N 8.14.

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

R = OCH_3; Ar=C_6H_5; m.p. 147–9 °C; yield 55%. – IR (KBr): υ = 3187 (NH), 1701, 1645 (C=O), 1560 (C=C), 1375, 1152 (SO_2), 1099, 1066, 1001 cm^{-1} (C=O=C). – ^{1}H NMR (DMSO-d_6) δ: 2.66–3.21 (m, 10H, piperidinyl), 3.91, 4.22 (2s, 6H, 2OCH_3), 7.00–7.09 (m, 5H, phenyl), 7.71, 7.91 (2d, 2H, CH=CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 9.71 (s, 1H, NH). – $C_{25}H_{26}N_2O_8S$(514.55): calcd. C 58.36; H 5.09; N 5.44; found C 58.11; H 5.22; N 5.26.

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

R = OCH_3; Ar = 3-indolyl; m.p. 91–3 °C; yield 45%. – IR (KBr): υ = 3280, 3181 (NH), 1699, 1671 (C=O), 1545 (C=C), 1371, 1150 (SO_2), 1111, 1009, 1000 cm^{-1} (C=O=C). – ^{1}H NMR (DMSO-d_6) δ: 2.61–3.22 (m, 10H, piperidinyl), 3.81, 4.11 (2s, 6H, 2OCH_3), 7.11–7.45 (m, 4H, indolyl), 7.73, 7.71 (2d, 2H, CH=CH), 7.98 (d, 1H, H-3), 8.12 (d, 1H, H-2), 8.35 (s, 1H, indolyl 2-H), 8.79 (s, 1H, NH), 10.20 (s, 1H, indolyl NH). – $C_{27}H_{27}N_3O_8S$(553.58): calcd. C 58.58; H 4.92; N 7.59; found C 58.31; H 4.86; N 7.45.

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 μL of methanolic solution of test compounds of 100 μg/mL was added to 2 mL of 6×10^{-5} 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 GmeH, Taufkirchen, 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, % $I = [(A_B - A_s)/A_B] \times 100$, where I = DPPH inhibition%, A_B = absorbance of control (t = 0 h) and A_S = absorbance of a tested sample at the end of the reaction (t = 1 h). All tests and analyses were done in triplicate and the results were averaged.

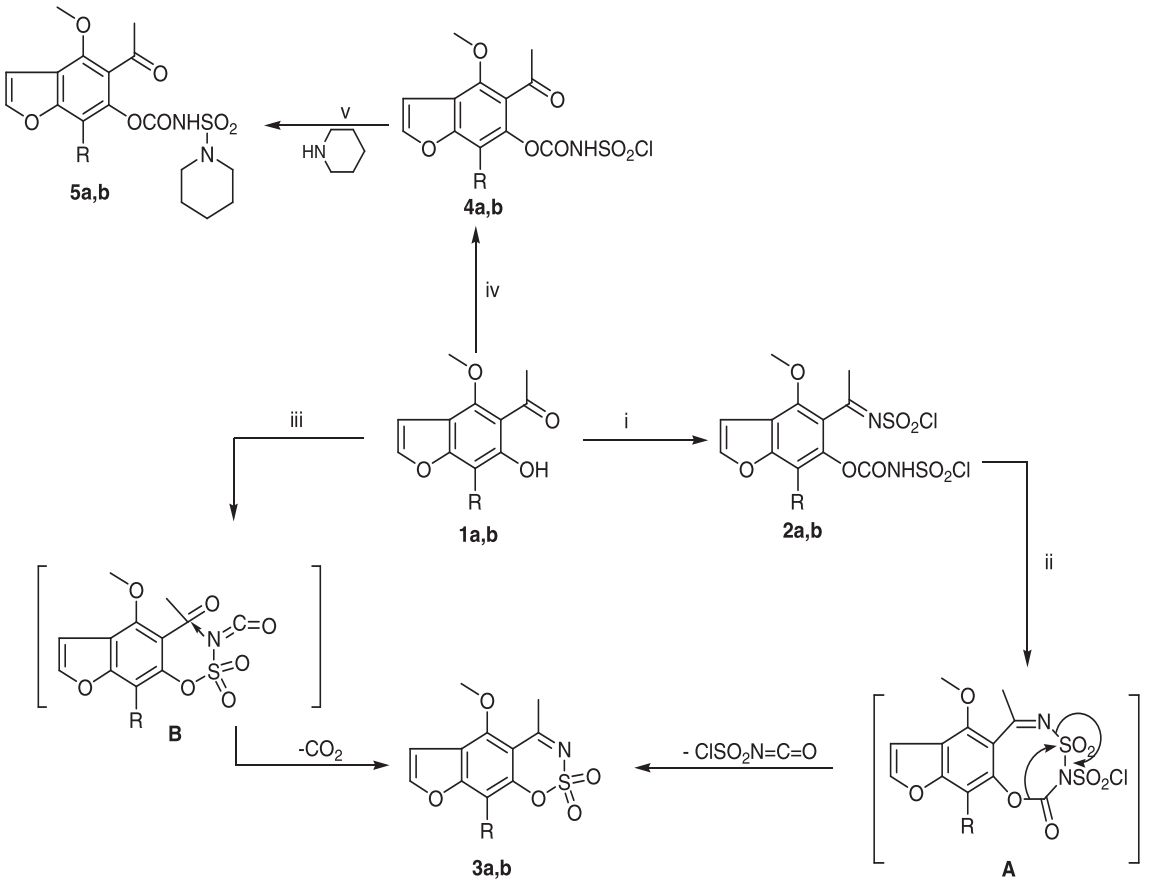
Cell culture

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 Biowahittkar, Belgium). All the media were supplemented with 1% antibiotic–antimycotic mixture (10,000 U mL^{-1} potassium penicillin, 10,000 μg/mL streptomycin sulfate, 25 μg/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, SG403INT, Sanford, ME, 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 μg/mL in DMSO) and incubated for 48 h. Medium was aspirated and 40 μL MTT salt (2.5 μg/mL) was added to each well and incubated for further 4 h. To stop the reaction and dissolve any formed formazan crystals, 200 μL 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 Adrinamycin® (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).



Scheme 1 Compounds **1**–**5**, R, **a** = H; **b** = OCH_3; reagents and conditions, (i) chlorosulfonyl isocyanate (CSI) (1:2 adduct), dry benzene, 0–5 °C; (ii) triethylamine, stirring; (iii) CSI (1:1 adduct), dry toluene, 100–105 °C; (iv) CSI (1:1 adduct), dry benzene, 0–5 °C; (v) dry1,4-dioxane, triethylamine, r.t.

Results and discussion

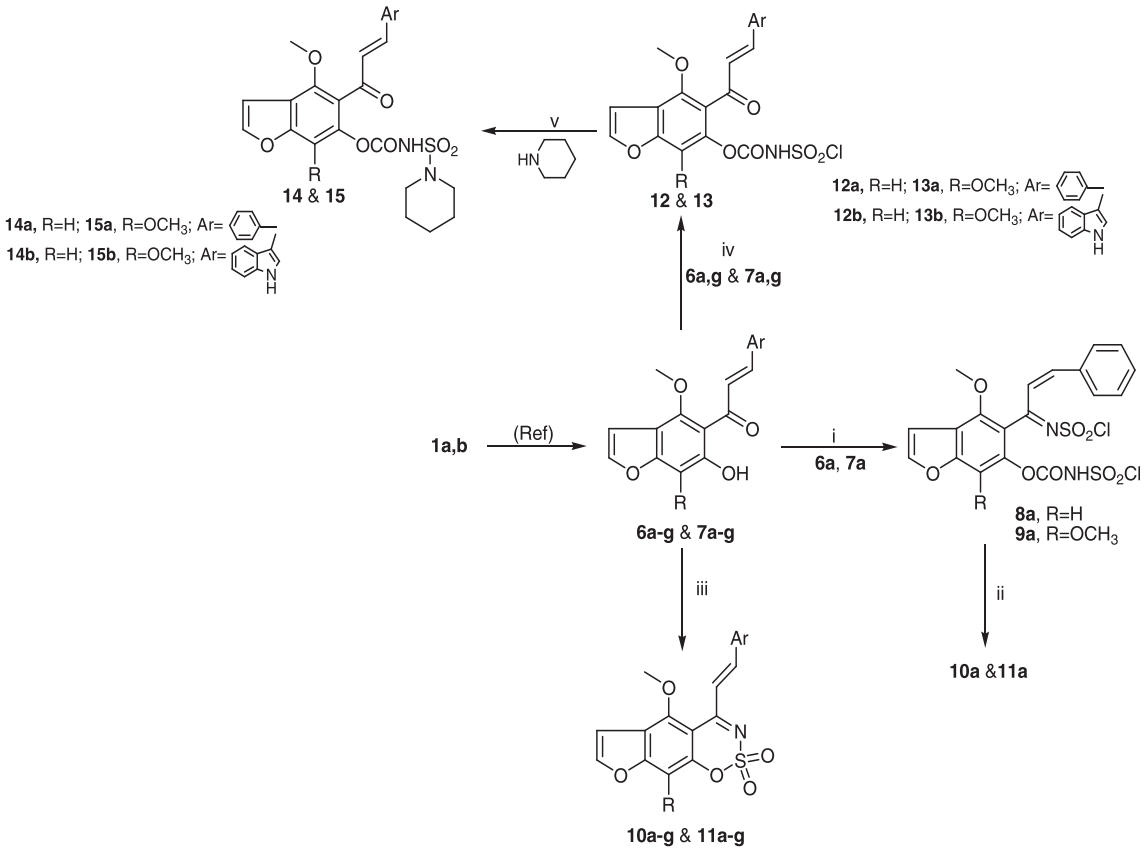
Chemistry

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)ethylidine) 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 ($ClSO_2NCO$) as reported in similar reactions [7] (Scheme 1).

On the other hand, cycloaddition reaction of CSI with compounds **1a** or **1b** in dry toluene at 100–105 °C (1:1 adduct) gave a product identical in all aspects (mp, TLC and spectra) with the cyclic 1,2,3-benzoxathiazine-7,7-dioxide derivatives **3a** and **3b** with good yield of 45% and 52%, respectively (method B). This reaction probably proceeded through the formation of aryloxyisocyanate **B** followed by intramolecular [2 + 2] addition of C═N group of the isocyanate to the C═O group of $COCH_3$ with subsequent loss of carbon dioxide as reported in similar reactions [7] (Scheme 1).

IR spectra of **3a** and **3b** showed no absorption bands for C═O and OH groups but showed absorption bands from 1135 to 1375 for SO_2 group (c.f. experimental section). The IR spectrum of compound **3a** as an example showed absorption bands at 1620, 1579, 1375, 1165, 1120, 1119, 1110 cm^{-1}. Its ^{1}HNMR spectrum revealed signals at 2.89 (s, 3H, CH_3), 4.40 (s, 3H, OCH_3), 6.77 (s, 1H, H-9), 7.62 (d, 1H, H-3), 8.22 ppm (d, 1H, H-2). Its ^{13}C NMR spectrum revealed signals at 18.5 (CH_3), 60.9 (OCH_3), 92.8–159.0 (Ar—C), 166.6 ppm (C═N).

Moreover, 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).



Scheme 2 Compounds **6** and **10**, R = H; Ar, **a** = C_6H_5, **b** = C_6H_4Cl-*p*, **c** = C_6H_4F-*p*, **d** = $C_6H_4OCH_3$-*p*, **e** = $C_6H_2(OCH_3)_3$-*3,4,5*, **f** = $C_6H_4N(CH_3)_2$-*p*, **g** = 3-indolyl; compounds **7** and **11**, R = OCH_3; Ar, **a** = C_6H_5, **b** = C_6H_4Cl-*p*, **c** = C_6H_4F-*p*, **d** = $C_6H_4OCH_3$-*p*, **e** = $C_6H_2(OCH_3)_3$-*3,4,5*, **f** = $C_6H_4N(CH_3)_2$-*p*, **g** = 3-indolyl; reagents and conditions; (i) chlorosulfonyl isocyanate (CSI) (1:2 adduct), dry benzene, 0–5 °C; (ii) triethylamine, stirring; (iii) CSI (1:1 adduct), dry toluene, 100–105 °C; (iv) CSI (1:1 adduct), dry benzene, 0–5 °C; (v) dry1,4-dioxane, triethylamine, r.t.

In a similar manner, α,β-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 over all 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). Also, α,β-unsaturated keto derivatives **6b–g** and **7b–g** allowed to react with CSI under the above mentioned conditions to give the corresponding 4-methoxy-5-arylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives (**10b–g**) and 4,9-dimethoxy-5-arylfuro[3,2-g]-1,2,3-benzoxathiazine-7,7-dioxide derivatives (**11b–g**), respectively (Scheme 2) with good yields ranging from 60% to 98%. IR (KBr) spectrum of **10a** as an example showed characteristic absorption bands at 1620, 1569, 1375, 1145, 1120, 1119 and 1110 cm^{-1}. Its ^1H NMR spectrum revealed signals at 4.32 (s, 3H, OCH_3), 6.88 (s, 1H, H-9), 7.01, 7.12 (2d, 2H, CH═CH), 7.32–7.68 (m, 5H, Ar—H), 7.90 (d, 1H, H-3), 8.12 ppm (d, 1H, H-2). Its ^{13}C NMR spectrum revealed signals at 61.2 (OCH_3), 96.3–155.2 (Ar—C), 159.3 ppm (C═N). Mass spectrum of **10a** showed molecular ion peak at m/z = 355 (53%) and base peak at m/z = 190.

On the other hand, reaction of compounds **6a, 6g**, **7a** or **7g** with CSI in dry benzene (1:1 adduct) at 0–5 °C afforded *N*-chlorosulfonyl carbamato-α,β-unsaturated keto derivatives **12a,b** and **13a,b**, respectively (Scheme 2). The reaction of the latter compounds **12a,b** and **13a,b** with piperidine in presence of triethylamine afforded the corresponding *N*-piperidinosulfonyl carbamates **14a,b** and **15a,b**, 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

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	17.4
11b	28.5
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.[a]	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%.

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 3 IC_{50} of the highly antiproliferative active compounds against human cancer cell lines.

Compd.	IC_{50} (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

IC_{50}. – Concentration required inhibiting cell viability by 50%.
IC_{50}. – > 0.78 is considered inactive.

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 IC_{50} 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 IC_{50} = 0.05 and 0.08 μmol/mL against A-549 and HCT-116, respectively, nearly as active as doxorubicin of IC_{50} = 0.04 and 0.06 μmol/mL, respectively.

On the other hand, compounds **11b** and **11c** showed higher activity with inhibition of IC_{50} = 0.03 and 0.02 μmol/mL, respectively, against A-549 compared to doxorubicin (IC_{50} = 0.04 μmol/mL). Also, compounds **11b** and **11c** showed higher activity with inhibition of IC_{50} = 0.05 and 0.03 μmol/mL, respectively, against HCT-116 compared to doxorubicin (IC_{50} = 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 **11b** and fluorine atom in **11c** at the *P*ara position of phenyl ring improved their activities toward A-549 and HCT-116 cancer cell lines.

Conclusions

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 *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 IC_{50} values ranging from 0.02 to 0.08 μmol/mL compared to doxorubicin of IC_{50} = 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.

References

[1] Dhar DN, Murthy KSK. Recent advances in the chemistry of chlorosulfonyl isocyanate. Synthesis 1986:437–49.
[2] Rasmussen JK, Hassner A. Recent development in the synthetic of chlorosulfonyl isocyanate. Chem Rev 1976;76:389–408.
[3] Suschitzky H, Walrond R, Hull R. Synthesis of heterocyclic compounds. Part 34: Reactions of chlorosulfonyl isocyanate with carbon–nitrogen double bonds. J Chem Soc Perkin Trans 1977;1:47–52.
[4] Dhar DN, Raghunathan R. Reaction of chlorosulphonyl isocyanate with 2-pyrazolines. Ind J Chem 1984;23B:1187–9.
[5] Kamal A, Sattur PB. Reactions with chlorosulfonyl isocyanate: formation of 2H–1,3-benzoxazine-2-ones. Synth Commun 1982;12:157–62.
[6] Hollwood F, Suschitzky H, Hull R. Chlorosulfonyl isocyanate addition to o-dialkylaminostyrenes: preparation of 6-(o-dialkylaminophenyl)-uracils. Synthesis 1982;2:662–5.
[7] Dhar DN, Bag AK. Synthesis of 4-styryl-1,2,3-benzoxathiazine 2,2-dioxides, naphtha[1,2-e]-1,2,3-oxathiazine 2,2-dioxide & 1,3-dipolar cycloaddition of 1,3-diphenylnitrilimine to 4-styryl-1,2,3-benzoxathiazine 2,2-dioxides. Ind J Chem 1983;22B:627–31.
[8] Liu J, Dumontet V, Simonin A, Iorga BI, Guerineau V, Litaudon MV, et al. Benzofurans from *Styrax agrestis* as acetylcholinesterase Inhibitors: structure activity relationships and molecular modeling studies. J Nat Prod 2011;74:2081–8.
[9] Rindhe SS, Rode MA, Karale BK. New benzofuran derivatives as an antioxidant agent. Indian J Pharm Sci 2010;72:231–5.
[10] Patil SL, Bhalgat CM, Burli S, Chithale SK. Synthesis, antibacterial and antioxidant properties of newer 3-(1-benzofuran-2-yl)-5-substituted aryl-1, 2-oxazole. Int J Chem Sci App 2010;1:42–9.
[11] Parekh S, Bhavsar D, Savant M, Thakrar S, Bavishi A, Parmar M, et al. Synthesis of some novel benzofuran-2-yl(4,5-dihyro-3,5-substituted diphenylpyrazol-1-yl) methanones and studies on the antiproliferative effects and reversal of multidrug resistance of human MDR1-gene transfected mouse lymphoma cells *in vitro*. Eur J Med Chem 2011;46:1942–8.
[12] El-Nakkady SS, Roaiah HF, El-Serwy WS, Soliman AM, El-Moez SI, Abdel-Rahman AA. Antitumor and antimicrobial activities of some hetero aromatic benzofurans derived from naturally occurring visnagin. Acta Pol Pharm 2012;69:645–55.
[13] Spath E, Gruber W. Natural chromones II. Constitution of visnagin (from Ammi visnaga). Ber Dtsch Chem Ges 1941;74B:1492–500.
[14] Schonberg A, Sina A. Khellin and allied compounds. J Am Chem Soc 1950;72:1611–5.
[15] Schonberg A, Sina A. On visnagin and khellin and related compounds. Simple synthesis of chromons. J Am Chem Soc 1950;72:3396–9.
[16] Schonberg A, Badran N, Strawkawksy N. Furo-chromones and coumarins. VII. Degradation of visnagin, khellin and related substances; experiments with chromic acid and hydrogen peroxide; and a synthesis of Eugenitin. J Am Chem Soc 1953;75:4992–5.
[17] Viuda-Martos M, El Gendy AE, Sendra E, Fernández-López J, Abd El Razik KA, Omer EA, et al. Chemical composition and antioxidant and anti-Listeria activities of essential oils obtained from some Egyptian plants. J Agric Food Chem 2010;58:9063–70.
[18] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 1983;65:55–63.