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Novel benzofuran derivatives: synthesis and antitumor activity

Abstract: A series of new benzofuran derivatives **3a–f**, **5a–e** containing a heterocyclic substituent linked to benzofuran nucleus at C-2 were synthesized as potential antitumor agents. These products were synthesized starting with 2-bromoacetylbenzofuran **1**. The structures of all compounds were established on the basis of analytical and spectral data. The synthesized compounds were tested against human liver carcinoma cell line (HEPG2) and all were more potent than the comparative standard (5-flurouracil). Compound **3f** was the most active (IC_{50} = 12.4 μg/mL).

Keywords: antitumor activity; benzofuran; HEPG2; one-pot reaction; thiazolidin-4-one.

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Introduction

Cancer has long been recognized as one of the most common causes of death [1]. Accordingly, many diverse strategies have been employed to develop new therapies or to improve existing treatments [2]. It is widely known that benzo[*b*]furan derivatives substituted at C-2 position show cytostatic and/or cytotoxic activity [3–5]. For example, ailanthoidol, a neolignan derivative, has been reported to have antiviral and antitumor activities [6] (Figure 1). In addition, salvinal, isolated from *Salvia miltiorrhiza*, showed inhibitory activity against tumor growth and induced apoptosis in human cancer cells [7] (Figure 1). Also, ebenfuran III [8] and moracins O [9] are 2-phenylbenzofurans derived from natural compounds exhibiting potent cytotoxic activities against human breast cancer and hepatocellular carcinoma. Recently, in the process of new drug discovery, the anticancer and antiviral activities of a variety of benzofuran derivatives I [10], NSC725612 and NSC725616 [4] have been reported (Figure 1). In addition, several reports have pointed out the value of some synthetic benzofuran derivatives, containing a heterocyclic substituent linked at C-2 of benzofuran nucleus, as antitumor agents. For example, compound A [11], compound B [12] and compound C [13] have been synthesized and evaluated as potent antitumor agents (Figure 1). Hence, in this study we have chosen salvinal, ebenfuran III and ailanthoidol as lead molecules to design and synthesize a series of several benzofuran derivatives substituted directly at C-2 with different heterocyclic groups. It has recently been shown that several thiazole [14] and thiazolidin-4-one [15] derivatives exhibit antitumor activity. Therefore, molecular combinations of the benzofuran system with both thiazole and thiazolidin-4-one rings were included in this work.

Results and discussion

Chemistry

The reaction of 2-acetylbenzofuran with bromine in a mixture of dioxane and ether afforded 1-(benzofuran-2-yl)-2-bromoethanone (**1**) according to a previously described method [16]. Treatment of **1** with thiosemicarbazide in dioxane gave 2-hydrazinothiazole derivative **2** in excellent yield of 80% [17]. The structure of compound **2** (Scheme 1) was confirmed by analysis of its ^{1}H NMR spectrum. 4-(1-Benzofuran-2-yl)thiazole-2-amine (**4** in Scheme 2) was synthesized in good yield by treatment of thiourea with 2-bromoacetylbenzofuran (**1**) in boiling ethanol [18].

The traditional method of the synthesis of thiazolidin-4-ones involves two steps, namely the formation of Schiff base followed by its cyclocondensation with thioglycolic acid [19, 20]. However, one of the major causes of waste in organic reaction chemistry is multi-stage synthesis. Thus, single-step syntheses are very attractive and possess high value in modern chemistry. Therefore, the novel single-step synthesis of compounds **3a–f, 5a–e** using a solid catalyst was performed. In this investigation, thiazolidin-4-ones **3a–f, 5a–e** were synthesized by molecular sieve – mediated one-pot three-component condensation reaction [21, 22] of an aromatic amine, an aromatic aldehyde and thioglycolic acid. The reaction was carried

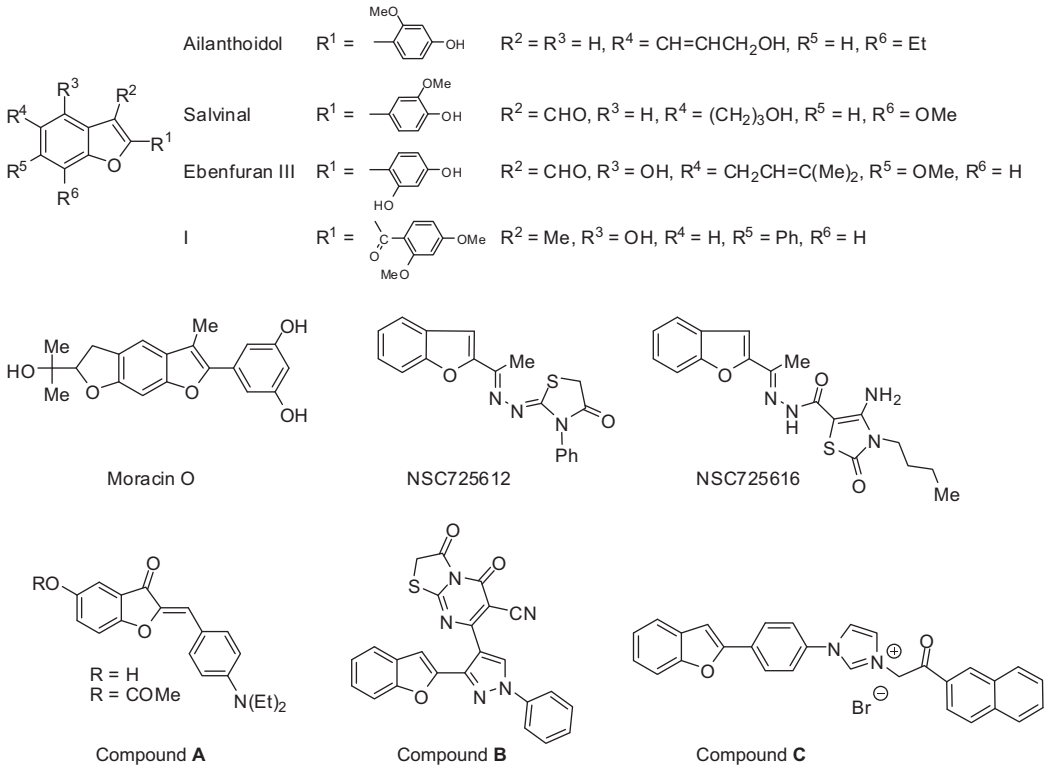
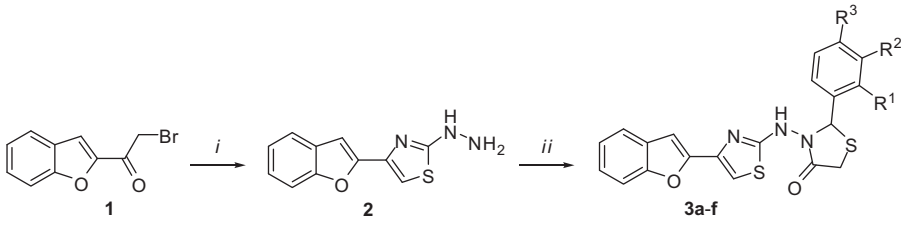
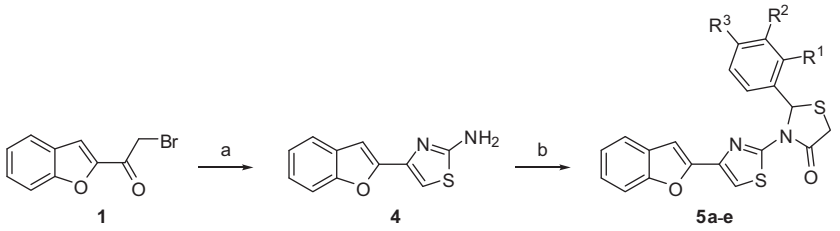


Figure 1 Structures of known 2-substituted benzofuran derivatives and thiazolidinone derivatives with potential antitumor activity.



3a: $R^1 = R^2 = H$, $R^3 = OH$
3b: $R^1 = OH$, $R^2 = R^3 = H$
3c: $R^1 = H$, $R^2 = R^3 = OMe$
3d: $R^1 = H$, $R^2 = OMe$, $R^3 = OH$
3e: $R^1 = R^2 = H$, $R^3 = OMe$
3f: $R^1 = R^2 = H$, $R^3 = N(Me)_2$

Scheme 1 Reagents and conditions: (A) $NH_2NHCSNH_2$, dry dioxane, stirring, rt, 3 h; (B) aldehyde, molecular sieves 4 Å, 1.1 equiv. thioglycolic acid, THF/toluene, Δ, 12–16 h.



3a: $R^1 = R^2 = H$, $R^3 = OH$
3b: $R^1 = OH$, $R^2 = R^3 = H$
3c: $R^1 = H$, $R^2 = R^3 = OMe$
3d: $R^1 = H$, $R^2 = OMe$, $R^3 = OH$
3e: $R^1 = R^2 = H$, $R^3 = N(Me)_2$

Scheme 2 Reagents and conditions: (A) NH_2CSNH_2, absolute ethanol, Δ, 5 h; (B) aldehyde, molecular sieves 4 Å, 1.1 equiv. thioglycolic acid, THF/toluene, Δ, 12–16 h.

out in toluene or THF as a solvent. The molecular sieves (zeolite, hydrated metal aluminosilicates) act mainly as a dehydrating agent [23, 24]. The reason for their success in catalysis is related to their high surface area and adsorption capacity.

Both compounds **2** and **4** were heated under reflux with different aromatic aldehydes and thioglycolic acid in THF or toluene as solvent and in the presence of molecular sieve as a catalyst. The thiazolidin-4-ones formation was found to be complete, as determined by TLC analysis, in approximately 12–16 h. Then, the molecular sieve was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was triturated with $NaHCO_3$ to remove any unreacted thioglycolic acid. It is worth noting that the use of THF as a solvent is preferred due to the observed decrease in reaction time and more facile work-up. The structures of thiazolidin-4-ones **3a–f, 5a–e** were assigned on the basis of their analytical and spectral data.

Biological activity

It has been found that a group of rubromycins and their analogs, a class of quinone antibiotics that possess a benzofuran system, strongly inhibit human telomerase enzyme, which leads to telomere shortening, arrest of cell growth and apoptosis [25]. Thus, benzofuran derivatives play an important role in inhibiting telomerase enzyme. All compounds **3a–f** and **5a–e** were subjected to preliminary screening, using single dose (500 µg/mL), for primary anticancer *in vitro* assay against HEPG2 (human liver carcinoma cell line), using the sulforhodamine-B (SRB) assay [26] (Table 1). Based on the requirement for cell line screening set by NCI that the percent growth of tumor cells (PG%) is 30% or less in at least one of the cell lines, it may

Table 1 Percent growth inhibition of compounds **3a–f** and **5a–e.**

		Growth inhibition
Compound	**% Survival (growth)**	**% Inhibition**
3a	41	59
3b	37	63
3c	38	62
3d	39	61
3e	39	61
3f	37	63
5a	38	62
5b	35	65
5c	35	65
5d	32	68
5e	35	65

Table 2 The effect of selected compounds on liver carcinoma cell lines (HEPG2) compared with standard drugs, doxorubicin and 5-fluorouracil.

Compound	**IC_{50} (µg/mL)**
Doxorubicin	3.73
5-Flurouracil	27.7
3b	17.8
3c	15.2
3f	12.4
5b	20.4
5c	16.8
5d	25
5e	17.8

IC_{50}: the dose of the compound which reduces survival to 50%.

be concluded that all these compounds are active because they approach this value, except **3a** which shows a PG% of 41% in HEPG2 cells at a concentration of 500 µg/mL.

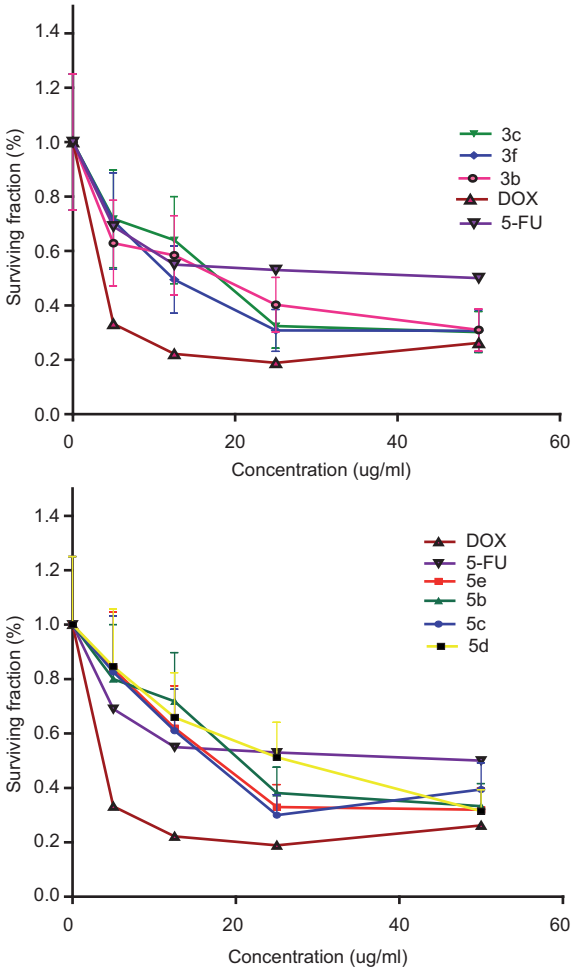


Figure 2 The standard curves for the most active compounds on HEPG2 liver cell lines compared with standard drugs, doxorubicin (DOX) and 5-flurouracil (5-FU).
Experiments were repeated three times and the results are displayed as mean values. Points are the means and bars are the SD values.

Table 3 Cytotoxicity on liver carcinoma cell lines (HEPG2) of the most active compounds with the SRB assay.

		Mean of surviving fraction±SD[a]						
Tumor cell line	**Compound conc. (μg/mL)**	**3b**	**3c**	**3f**	**5b**	**5c**	**5d**	**5e**
HEPG2	0.000	1.00±0.25	1.00±0.25	1.00±0.25	1.00±0.25	1.00±0.25	1.00±0.25	1.00±0.25
	5.000	0.62±0.15	0.71±0.179	0.71±0.177	0.8±0.2	0.82±0.206	0.84±0.21	0.83±0.209
	12.500	0.58±0.14	0.64±0.16	0.49±0.12	0.72±0.18	0.61±0.15	0.65±0.16	0.62±0.155
	25.000	0.40±0.10	0.32±0.08	0.31±0.077	0.38±0.09	0.3±0.075	0.51±0.13	0.33±0.082
	50.000	0.31±0.077	0.30±0.075	0.307±0.07	0.33±0.083	0.39±0.098	0.32±0.078	0.32±0.08

[a]Mean of surviving fraction±SD: mean of three assays±standard deviation.

In addition, further experiments were performed using different concentrations to calculate IC_{50} values of the most active compounds in comparison with doxorubicin and 5-flurouracil as positive controls. The cytotoxic activity of the tested compounds on HEPG2 were expressed as IC_{50} (Table 2), where IC_{50} (μg/mL) is the dose of compound which reduces survival to 50%. The relation between the surviving fraction and drug concentration is plotted to get the survival curve of the tumor cell line (Figure 2).

Structure-activity relationship

The structure-activity correlation of the obtained results revealed that the presence of the NH spacer between the thiazolidinone ring and the thiazolylbenzofuran system may be essential for biological activity. Interestingly, the presence of such a spacer in the **3 a–f** series plays an important role in increasing antitumor activity ($IC_{50} \leq 17.8$ μg/mL). Compound **3f** is the most potent agent with IC_{50} = 12.4 μg/mL. Furthermore, the results suggest that the different functional groups on the benzene ring, with different hydrophilic properties, also contribute to biological activity. The derivatives **3c** and **5c**, with two methoxy groups at the ortho and para positions show good antitumor activity. Moreover, the dimethylamino group at the para position of **3f** and **5e** appears to improve biological activity to some extent. By contrast, compounds **3b** and **5b** display moderate activity ($IC_{50} \geq 17.8$ μg/mL), indicating the effect of the hydroxy group at the ortho position. In addition, the presence of the hydroxy group in the para position of **3a** and **5a** dramatically reduces the growth inhibitory potential.

Conclusions

All tested compounds show remarkable antitumor activity against human HEPG2 cell lines as they induce a reasonable growth inhibition in a dose-dependent manner. Within the **3a–f** series, compound **3f** is the most potent and the activity of the remaining compounds is in the order **3a** < **3e** = **3d** < **3b** < **3c**. For the **5a–e** series, the activity order is **5a** < **5d** < **5b** < **5e** < **5c**.

Experimental

Melting points were recorded using a Fisher-John apparatus and are uncorrected. Elemental analyses were performed at the Microanalytical Unit, Cairo University, Egypt. IR spectra were recorded on a Mattason 5000 FT-IR spectrometer in KBr discs. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Ac 400 FT NMR spectrometer in DMSO-d_6, at Georgia State University, Atlanta, GA, USA. Mass spectra were obtained on a JOEL JMS-600H spectrometer at Cairo University, Egypt. The intermediate 1-(benzofuran-2-yl)-2-bromoethanone (**1**) was prepared according to a previously described method [16].

1-(Benzofuran-2-yl)-2-bromoethanone (1) To a solution of 2-acetylbenzofuran (16.0 g, 0.1 mol) in dioxane (50 mL) and ether (75 mL), bromine (15.9 g, 0.1 mol) was added gradually over 1.5 h at 20–30°C. Then the mixture was diluted with ethanol (20 mL) and heated under reflux for 30 min. The resulting colorless solution was poured into water (300 mL). The yellow crystalline precipitate was collected, washed with water, dried at 40–50°C and crystallized from ethanol/water (9:1) to give 22.9 g (86%) of **1**; mp 89–90°C [16].

2-(2-Hydrazinylthiazol-5-yl)-benzofuran(2) A mixture of 2-bromoacetylbenzofuran (0.48 g, 2 mmol) and thiosemicarbazide (0.182 g, 2 mmol) in dry dioxane (10 mL) was stirred for 2–3 h at room temperature. The separated crystals were filtered, washed with ethanol, air dried and crystallized from ethanol: yield 80%; mp 176–179°C [17]; ^{1}H NMR: δ 10.28 (s, 1H, NH-), 9.40 (s, 2H, NH_2, D_2O exchangeable), 7.16 (s, 1H, C3″-H), 7.19 (s, 1H, C4-H), 7.23–7.45 (m, 2H, C5′-H, C6′-H), 7.61 (d, 2H, C4′-H, J = 7 Hz), 7.75 (d, 2H, C7′-H, J = 7 Hz); ^{13}C NMR: 21.2 (C-S), 111.1 (C3′-benzofuran), 111.6 (C7′-benzofuran), 122.1 (C4′-benzofuran), 123.4 (C5′-benzofuran), 126.7 (C6′-benzofuran), 127.0 (C3″-benzofuran), 141.6 (C5-thiazole), 148.0 (C2′-benzofuran), 154.7 (C1″-benzofuran), 163.4 (C2-thiazole).

General method for the synthesis 3-(4-(benzofuran-2-yl)thiazol-2-ylamino)-2-(substituted phenyl)thiazolidin-4-ones 3a–f

2-(2-Hydrazinylthiazolyl)benzofuran (0.5 g, 1 mmol) and an aromatic aldehyde (1 mmol) were stirred in THF for 5 min, followed by addition of mercaptoacetic acid (1.1 mmol) and molecular sieves 4 Å. The mixture was stirred under reflux, until TLC showed disappearance of the starting materials. The molecular sieves were removed by filtration. The filtrate was concentrated *in vacuo* and the residue was triturated with 5% $NaHCO_3$. The crude product **3a–f** was filtered and crystallized from petroleum ether/diethyl ether (2:1).

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(4-hydroxyphenyl) thiazolidin-4-one (**3a**) Yield 0.14 g (84%); mp 155°C; IR: 748, 1511, 1567, 1627, 2931, 3137, 3401 cm^{-1}; ^1H NMR: δ 3.65 (s, 1H, br OH), 3.73 (s, 2H, C5-H), 4.21 (br s, 1H, NH), 6.76 (s, 1H, C2-H), 7.39–8.02 (m, 10H, Ar-H); MS: m/z 410 (16.10, M^++1), 409 (15, M^+), 231 (100), 143 (74). Anal. Calcd for $C_{20}H_{15}N_3O_3S_2$ (409.48): C, 58.66; H, 3.69; N, 10.26. Found: C, 58.35; H, 3.55; N, 10.18.

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(2-hydroxyphenyl) thiazolidin-4-one (**3b**) Yield 0.108 g (72%); mp 145–147°C; IR: 740, 1511, 1567, 1627, 2931, 3137, 3401 cm^{-1}; ^1H NMR: δ 3.65 (s, 1H, br OH); 3.73 (s, 2H, C5-H); 4.21 (br s, 1H, NH), 6.76 (s, 1H, C2-H), 7.39–8.02 (m, 10H, Ar-H); MS: m/z 409 (13, M^+), 198 (100), 175 (25). Anal. Calcd for $C_{20}H_{15}N_3O_3S_2$ (409.48): C, 58.66; H, 3.69; N, 10.26. Found: C, 58.45; H, 3.36; N, 10.19.

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(3,4-dimethoxyphenyl)thiazolidin-4-one (**3c)** Yield 0.116 g (70%); mp 110–112°C; IR: 685, 1516, 1585, 1679, 2929, 3131 cm^{-1}; ^1H NMR: δ 3.80 (s, 2H, C5-H), 3.90 (s, 6H, $2OCH_3$), 7.05–7.70 (m, 9H, Ar-H), 8.00 (s, 1H, C2-H); MS: m/z 454 (58, M^+), 379 (11), 216 (100). Anal. Calcd for $C_{22}H_{19}N_3O_4S_2$ (453.53): C, 58.26; H, 4.22; N, 9.27. Found: C, 58.13; H, 4.40; N, 9.17.

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(4-hydroxy-3-methoxyphenyl) thiazolidin-4-one (3d) Yield 0.104 g (65%); mp 160°C; IR: 710, 1520, 1570, 1627, 2931, 3137, 3401 cm^{-1}; ^1H NMR: δ 3.52 (s, 1H, br OH); 3.75 (s, 2H, C5-H); 3.90 (s, 3H, OCH_3); 6.90–7.89 (m, 10H, 9Ar-H, C2-H); MS: m/z 440 (1.32, M^+), 216 (100), 174 (46). Anal. Calcd for $C_{21}H_{17}N_3O_4S_2$ (439.51): C, 57.39; H, 3.90; N, 9.56. Found: C, 57.23; H, 3.88; N, 9.31.

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(4-methoxyphenyl) thiazolidin-4-one (3e) Yield 0.085 g (55%); mp 156–158°C; IR: 1002, 1509, 1585, 1679, 2929, 3131 cm^{-1}; ^1H NMR: δ 3.77 (s, 2H, C5-H), 3.80 (s, 3H, OCH_3), 6.68–7.69 (m, 11H, 10Ar-H, C2-H); MS: m/z 424 (2, M^+), 43 (100). Anal. Calcd for $C_{21}H_{17}N_3O_3S_2$ (423.51): C, 59.56; H, 4.05; N, 9.92. Found: C, 59.43; H, 3.98; N, 9.71.

3-(4-(Benzofuran-2-yl)thiazol-2-ylamino)-2-(4-(dimethylamino) phenyl)thiazolidin-4-one (**3f)** Yield 0.119 g (45%); mp 96–98°C; IR: 680, 1510, 1580, 1685, 2930, 3130 cm^{-1}; ^1H NMR: δ 3.87 (s, 2H, C5-H), 3.78 (s, 6H, N $(CH_3)_2$), 6.68–7.69 (m, 11H, 10Ar-H, C2-H); MS: m/z 437 (1, M^++1), 436 (1, M^+), 145 (100), 118 (76). Anal. Calcd for $C_{22}H_{20}N_4O_2S_2$ (436.55): C, 60.53; H, 4.62; N, 12.83. Found: C, 59.99; H, 4.38; N, 12.61.

Synthesis of 4-(benzofuran-2-yl) thiazol-2-amine (4)

A solution of 2-bromoacetylbenzofuran (1.35 g, 0.01 mol) and thiourea (0.4 g, 0.01 mol) in ethanol (10 mL) was heated under reflux for 3 h and then neutralized with 10% K_2CO_3. The separated solid was filtered, dried and crystallized from ethanol/water (9:1) [27]: yield 1.1 g (90%); mp 214°C; ^1H NMR: δ 6.99 (s, 2H, NH_2, D_2O exchangeable), 7.05 (s, 1H, C3′-H), 7.20–7.35 (m, 3H, C5′-H, C6′-H and C4-H), 7.55 (d, 1H, C4′-H, *J* = 8 Hz); 7.65 (d, 1H, C7′-H, *J* = 8Hz).

General procedure for the synthesis of 3-(4-(benzofuran-2-yl)thiazol-2-yl)-2-(substituted phenyl)-thiazolidin-4-ones 5a–e

A solution of 2-(2-aminothiazolyl)benzofuran (1.2 g, 1 mmol) and an aromatic aldehyde (1 mmol) in toluene or THF was stirred and treated with mercaptoacetic acid (1.1 mmol) and molecular sieves 4 Å. The mixture was stirred under reflux until TLC showed disappearance of starting materials. The molecular sieves were removed by filtration. The filtrate was concentrated *in vacuo* and the residue was triturated with 5% $NaHCO_3$. The crude product **5a–e** was filtered and crystallized from petroleum ether/diethyl ether (2:1).

3-(4-(Benzofuran-2-yl)thiazol-2-yl)-2-(4-hydroxyphenyl)thiazolidin-4-one (**5a**) Yield 0.177 g (88%); mp 125°C; IR: 770, 1511, 1567, 1627, 2931, 3401 cm^{-1}; ^1H NMR: δ 3.65 (s, 1H, br OH), 3.99 (s, 2H, C5-H), 6.76 (s, 1H, C2-H), 7.20–7.89 (m, 10H, Ar-H); MS: m/z 395 (1.42, M^++1), 31 (100), 215 (50). Anal. Calcd for $C_{20}H_{14}N_2O_3S_2$ (394.47): C, 60.90; H, 3.58; N, 7.10. Found: C, 59.10; H, 3.55; N, 7.19.

3-(4-(Benzofuran-2-yl)thiazol-2-yl)-2-(2-hydroxyphenyl)thiazolidin-4-one (**5b**) Yield 0.103 g (57%); mp 140–142°C; IR: 687, 1518, 1580, 1660, 2920, 3405 cm^{-1}; ^1H NMR: δ 3.99 (s, 2H, C5-H), 7.00–7.70 (m, 10H, Ar-H), 10.80 (s, 1H, br OH), 10.20 (s, 1H, C2-H); MS: m/z 395 (0.4, M^++1), 394 (0.5, M^+), 63 (100), 78 (95). Anal. Calcd for $C_{20}H_{14}N_2O_3S_2$ (394.47): C, 60.90; H, 3.58; N, 7.10. Found: C, 60.88; H, 3.65; N, 7.16.

3-(4-(Benzofuran-2-yl)thiazol-2-yl)-2-(3,4-dimethoxyphenyl)thiazolidin-4-one (**5c**) Yield 0.13 g (65%); mp 98–100°C; IR: 1001, 1511, 1589, 1683, 2925 cm^{-1}; ^1H NMR: δ 3.78 (s, 6H, $2OCH_3$), 3.87 (s, 2H, C5-H), 7.05–7.89 (m, 9H, Ar-H), 9.66 (s, 1H, C2-H); MS: m/z 439 (15, M^++1), 438 (52, M^+), 151 (100), 165 (90). Anal. Calcd for $C_{22}H_{18}N_2O_4S_2$ (438.52): C, 60.26; H, 4.14; N, 6.39. Found: C, 60.44; H, 4.28; N, 6.59.

3-(4-(Benzofuran-2-yl)thiazol-2-yl)-2-(4-hydroxy-3-methoxyphenyl)thiazolidin-4-one (5d) Yield 0.107 g (55%); mp 110–112°C; IR: 710, 1525, 1567, 1627, 2929, 3401 cm^{-1}; ^1H NMR: δ 3.52 (s, 1H, br OH), 3.77 (s, 2H, C5-H), 3.90 (s, 3H, OCH_3), 6.90–7.89 (m, 10H, 9Ar-H, C2-H); MS: m/z 424 (1, M^+), 43 (100), 78 (30). Anal. Calcd for $C_{21}H_{16}N_2O_4S_2$ (424.49): C, 59.42; H, 3.80; N, 6.60. Found: C, 59.64; H, 3.68; N, 6.89.

3-(4-(Benzofuran-2-yl)thiazol-2-yl)-2-(4-(dimethylamino)phenyl)thiazolidin-4-one (5e) Yield 0.148 g (76%); mp 116–117°C; IR: 690, 1550, 1569, 1663, 2930 cm^{-1}; ^1H NMR: δ 3.87 (s, 2H, C5-H), 3.78 (s,

6H, $N(CH_3)_2$), 6.68–7.69 (m, 11H, 10Ar-H, C2-H); MS: m/z 422 (3, M^++1), 421 (2, M^+), 45 (100), 134 (15). Anal. Calcd for $C_{22}H_{19}N_3O_2S_2$ (421.54): C, 62.68; H, 4.54; N, 9.97. Found: C, 62.84; H, 4.88; N, 9.69.

Sulforhodamine-B (SRB) assay of cytotoxicity

The aim of the present study was to analyze the effect of newly synthesized compounds on HEPG2 cells in comparison with doxorubicin and 5-flurouracil as positive controls. Human tumor cell lines were obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection, RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA). The medium was prepared and used for culturing and maintenance of the human tumor cell lines. The prepared medium was kept in a refrigerator at 4°C and checked at regular intervals for contamination. Before use, the medium was warmed at 37°C in a water bath and supplemented with penicillin/streptomycin and FBS. Experiments were set up using the SRB assay. Cells were plated in 96-multiwell plates (5 × 104–105cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compounds tested (0, 5, 12.5, 25 and 50 μg/mL) were added to the cell monolayer. Each concentration was evaluated three times and each dose was incubated with the cells in three different wells. Monolayer cells were incubated with the compounds for 48 h at 37°C and in an atmosphere of 5% CO_2. Control cells were treated with vehicle alone. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris(hydroxymethyl)aminomethane] for determination of optical density (OD). The OD of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter Tech. Σ 960, USA). The mean background absorbance was automatically subtracted and a mean value of each drug concentration was calculated. The average values of three calculations are presented as mean±SD (standard deviation), (Table 3). The percentage of cell survival was calculated as follows: survival fraction = OD (treated cells)/OD (control cells).

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