Synthesis of New 4-Oxo-thiazolidine-5-ylidenes of Antitumor and Antioxidant Activities

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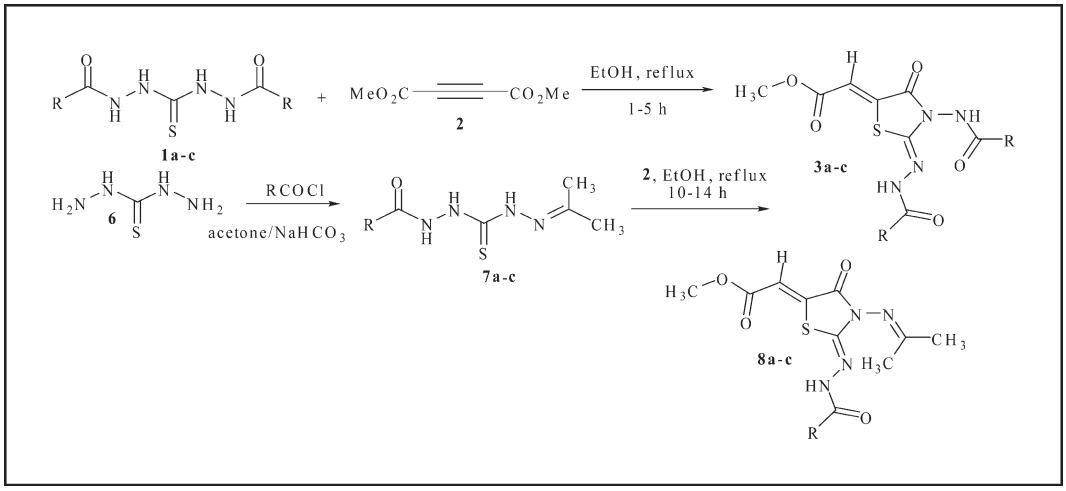
Received August 11, 2009
DOI 10.1002/jhet.290
Published online 14 April 2010 in Wiley InterScience (www.interscience.wiley.com).



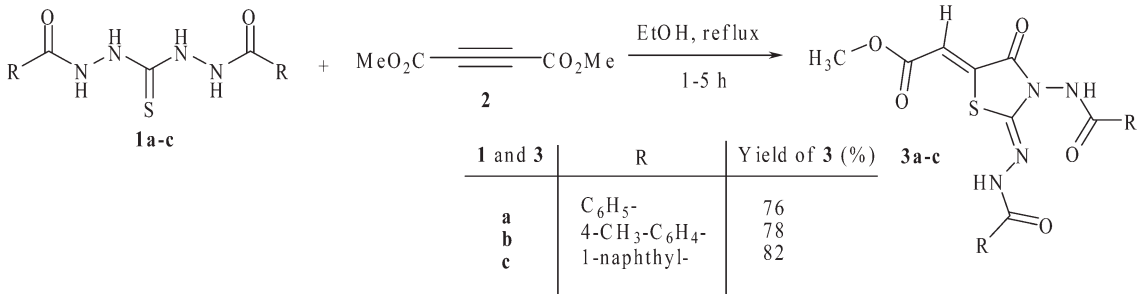
Reaction of diacyl thiocarbohydrazides with dimethyl but-2-ynedioate in refluxing ethanol led to 4-oxa-thiazolidine-5-ylidene-acetates in good yields. Reaction of the newly prepared *N*-(2-(propan-2-ylidene) hydrazine-carbonothioyl)arylhydrazides with dimethyl but-2-ynedioate gave the corresponding (*Z*)-methyl-2-arylhydrazide-4-oxo-3-(propan-2-ylideneamino)thiazolidine-5-ylidene)-acetates. The mechanism is discussed. Antitumor and antioxidant activities have been also investigated.

J. Heterocyclic Chem., **47**, 547 (2010).

INTRODUCTION

The development of simple synthesis routes for widely used organic compounds from readily available reagents is one of the major tasks in organic synthesis. Recent report has shown that various *N*-ethyl hydrazine-carbothioamides can undergo different cyclization reactions to give five member heterocycles, which showed a general stimulation effect on B cell's response [1]. Thiazolidine-4-one ring systems are known to possess antibacterial [2,3], antituberculosis [4–6], antiviral [7–14], anticancer [15–18], and antioxidant [19]. In view of the various physiological activities of thiazolidinones, many thiazolidinone derivatives have been prepared. 4-Phenyl-thiosemicarbazide reacts smoothly with dimethyl but-2-ynedioate in the presence of aldehydes or ketones under solvent free conditions to produce highly functionalized thiazolidine-4-ones [20]. The reaction of thioureas with acetylenic esters has been reported to give a thiazolin-4-one, an imidazolinthion, or a 1,3-thiazin-4-one [21]. Recent reports by Aly *et al*. [22] demonstrated that the reaction of *N*-aroyl thioureas with of dimethyl but-2-ynedioate under reflux in acetic acid yielded the corresponding 1,3-thizinones. Additionally, diethyl maleate reacts with *N*-substituted-hydrazino-carbothioamides to form ethyl [1,2,4]triazolo[3,4-*b*][1,3]thiazine-5-carboxylates [23]. Reaction proceeds *via* bicyclization and oxidation processes [23]. Whilst 2,3-diphenylcyclopropenone reacts with ylidene-*N*-phenylhydrazine-carbothioamides to form the pyrrolo[2,1-*b*]-1,3,4-oxadiazoles *via* formal [2 + 3]cycloaddition [24]. On the other side, we reported on one pot synthesis of 1,3-thiazin-2-ylidene-substituted hydrazides *via* one-pot reaction of *N*-substituted-hydrazino-carbothioamides with 1,4-diphenylbut-2-

Scheme 1. Synthesis of new 1,3-thiazolidine-4-ones **3a–c**.



1 and 3	R	Yield of 3 (%)
a	C_6H_5-	76
b	4-CH_3-C_6H_4-	78
c	1-naphthyl-	82

yne-1,4-dione [25]. On the basis of aforementioned encouraged results, we investigate the reaction of acyl thiocarbohydrazides with dimethyl but-2-ynedioate. Moreover antitumor and antioxidant activities of the isolated products have been investigated.

RESULTS AND DISCUSSION

Chemistry. We have now reacted diacyl thiocarbohydrazides **1a–c** [26] with dimethyl but-2-ynedioate (**2**); the reactions gave mainly the corresponding (*Z*)-methyl-2-[(*Z*)-3-aroylamido-2-(2-arylhydrazono)]-4-oxa-thiazolidine-5-ylidene)-acetates (**3a–c**, Scheme 1). For structure prevalent, we choose one derivative identified as **3b** and investigate its NMR in comparative with its expected regioisomers **3bI–III** (Fig. 1). As IR and ^{13}C NMR did not reveal any absorbance of the C=S group. Moreover, the five C=S in ^{13}C chemical shifts are all too far upfield for a C=S. Therefore the upfield five carbon signals in the ^{13}C NMR spectra of compound 3b must represent three carbon signals of four C=O and one for the C=N carbon (see the EXPERIMENTAL SECTION). Accordingly the structure of the regioisomer **3bI** is excluded. The magnitude of the coupling constant (J = 5.2 Hz) further argues that ring carbonyl (C-4) and vinylic-proton are mutually *cis*. Under gated decoupling, the ring carbonyl (C-4) couples to vinylic-proton with J = 5.2 Hz, a value which requires a three- not two-bond coupling as depicted in structures **3b** and **3bIII** and excluded the formation of other regioisomers (**3bI** and **3bII**). It was reported, if the coupling constant for the vinylic-proton and endocyclic carbon atom in a condensation product is about ~5 Hz (*vicinal*-coupling), this product has a five-membered ring; if the coupling constant approaches a value of 1 Hz (*geminal*-coupling), the product should be assigned six-membered thiazine structure [27,28]. Most of the C—H coupling constants are within conventional ranges [29], except that the J_{C-H} values for C-2′ and C-3′ are unusually small for benzenes. Presumably this arises from restricted rotation. In compound **3b** there are two *p*-toluoyl units, one slightly broadened, which is presumably due to restricted rotation. Toluamide rotation and NH exchange are independent processes, which in general occur at different rates. The methoxyl protons are distinctive at δ_H = 3.81; this signal gives HMQC correlation with the attached carbon at δ_C = 52.7 and HMBC correlation with the ester carbonyl at δ_C = 165.6. The signal (δ_C = 160.9) giving HMBC correlation to vinylic-H (δ_H = 6.96) is assigned as C-4. The carbon (δ_C = 116.8) giving HMQC correlation to vinylic-H is assigned as

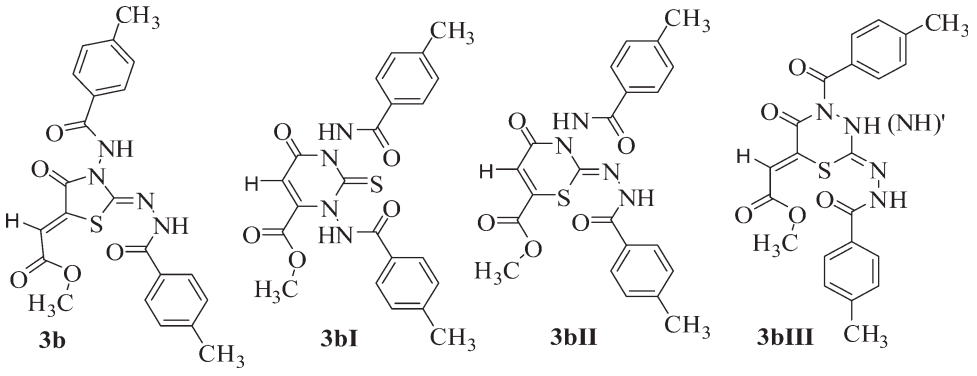


Figure 1. Structure of some commercial triazolopyrimidine-2-sulfonamide herbicides.

Table 1

NMR spectroscopic data of compound **3b**.

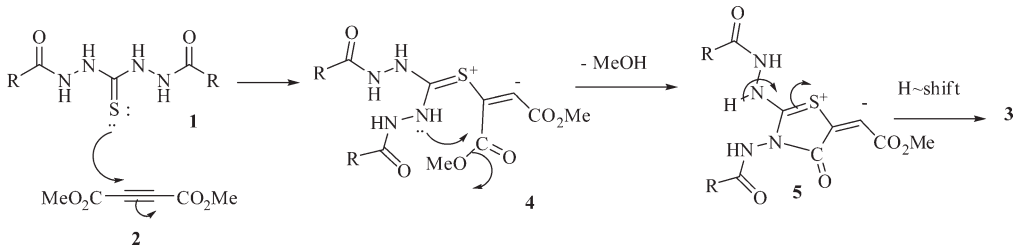
	COSY	HMQC	HMBC	Assignment
^{1}H NMR (ppm)				
11.67 (bs; 1H)	11.34			benzamido-NH
11.34 (bs; 1H)	11.67			hydrazono-NH
7.88 (d, J = 7.7; 2H)	7.39			H-2′
7.77 (bd, J = 6.4; 2H)	7.31			H-2″
7.39 (d, J = 8.0; 2H)	7.88			H-3′
7.31 (bd, J = 6.1; 2H)	7.77			H-3″
6.96 (s; 1H)				vinylic-H
3.81 (s; 3H)				OCH_3
2.41 (s; 3H)				benzamido-CH_3
2.37 (s; 3H)				hydrazono-CH_3
^{13}C NMR (ppm)				
165.6 (q, J = 4.3)			3.81	ester C=O
164.3 (dt, J_d = 8.6, J_t = 4.2)			7.88	benzamido-C=O
163.4 (b)			11.34	hydrazono-C=O
160.9 (d, J = 5.2)			6.96	C-4
152.0 (b)			11.34	C-2
143.0 (q, J = 7.5)			7.88, 2.41	C-4′
141.8 (bq)			7.77, 2.37	C-4″
137.5 (s)			6.96	C-5
129.8 (t, J = 7.7)				C-1′
129.2 (ddq, J_d = 165.8, 5.5; J_q = 5.5)		7.39	7.39, 2.41	C-3′
128.9 (bd, J = 136.9)		7.31	2.37	C-3″
127.8 (t, J = 7.6)				C-1″
127.7 (dd, J = 160.9, 6.4)		7.88	7.88	C-2′
127.5 (bd, J = 136.3)		7.77		C-2″
116.8 (d, J = 173.7)		6.96		vinylic-CH
52.7 (q, J = 148.2)		3.81		OCH_3
21.0 (tq, J_t = 4.9, J_q = 126.7)		2.41	7.39	benzamido-CH_3
20.9 (tq, J_t = 4.9, J_q = 126.7)		2.37		hydrazono-CH_3

vinylic-CH. One other carbon (δ_C = 137.5) gives HMBC correlation to vinylic-H, and is assigned as C-5. The benzamido- and hydrazono-C=O appear distinctively at δ_C = 164.3 and 163.4, respectively. They give HMBC correlation to the ortho protons on the attached tolyl rings at δ_H = 7.88 and 7.77, respectively.

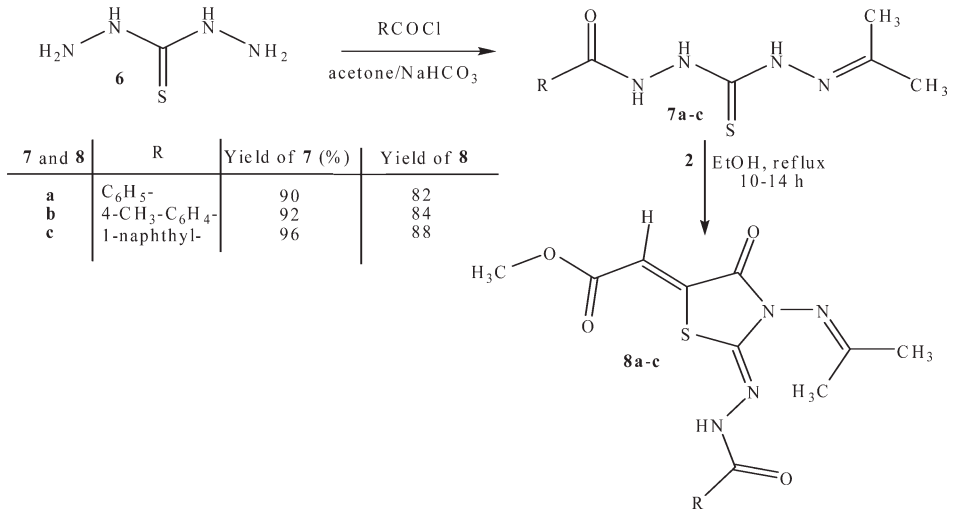
The signals at δ_C = 143 and 141.8 give HMBC correlation with the *ortho* protons and CH_3 (δ_H = 7.88, 2.41 and 7.77, 2.37) are assigned as C-4′ and C-4″, respectively. The assignment of distinctive hydrogen and carbon signals and their δ values as well as the corresponding coupling constants of compound **3b** are as shown in Table 1.

On the basis of well established chemistry of electrophilic acetylenes [21], it is reasonable to assume that compounds **4** resulted from the initial conjugate addition of the sulfur atom of **2** to the acetylenic ester. Then, the ester group of intermediate **4** was attacked by the amino moiety to yield **5** by elimination of methanol molecule (Scheme 2). Hydrogen shift is then proposed to be

Scheme 2. Plausible mechanism of 1,3-thiazolidine-4-ones **3a–c**.



Scheme 3. Synthesis of 1,3-thiazolidine-4-ones **8a–c**.



7 and 8	R	Yield of 7 (%)	Yield of 8
a	C_6H_5-	90	82
b	4-CH_3-C_6H_4-	92	84
c	1-naphthyl-	96	88

occurred in **5** to produce the stable heterocycle **3** (Scheme 2). Previously it was reported that thiocarbohydrazide (**6**) condensed with acetone to form the corresponding mono-condensed products [30] likewise in case of **7a–c** (Scheme 3). Herein we reacted compound **2** with aroyl chlorides in presence of acetone. The reaction proceeds successfully to give compounds **7a–c** in good yields (Scheme 3). Interestingly, on reacting the newly prepared compounds **7a–c** with dimethyl but-2-ynedioate ethyl ester (**2**), the reaction gave the corresponding thiazolidines **8a–c** in good yields (Scheme 3).

In compound **8a**, the ^{1}H NMR spectrum showed the two methyl protons are distinctive at $\delta_H = 2.06$ and 1.94; this signals gives HMQC correlation with the attached carbon at $\delta_C = 25.0$ and 18.7 and HMBC correlation with the carbon at $\delta_C = 168.9$ which is assigned as $C(CH_3)_2$. The methoxyl protons are distinctive at $\delta_H = 3.87$; this signal gives HMQC correlation with the attached carbon at $\delta_C = 52.6$ and HMBC correlation with the ester carbonyl at $\delta_C = 166.1$. The signal ($\delta_C = 162.1$) giving HMBC correlation to vinylic-H ($\delta_H = 6.96$) is assigned as C-4. The carbon ($\delta_C = 117.3$) giving HMQC correlation to vinylic-H is assigned as vinylic-CH. One other carbon ($\delta_C = 139.4$) gives HMBC correlation to vinylic-H, and is assigned as C-5. The benzoyl C=O appear at $\delta_C = 166.0$ gives HMBC correlation with *ortho* protons at ($\delta_H = 7.91$).

Biological section.

Cytotoxicity against Hep-G2 cells. Using MTT assay, we studied the effect of the compounds on the proliferation of human hepatocellular carcinoma after 48 h incubation. Incubation of Hep-G2 cell line with gradual doses of the compounds led to insignificant change in the growth of Hep-G2 cells as indicated from their IC_{50} values (>100 μ*M*), except, compound **3c**, which resulted in a high inhibition of the cell growth of Hep-G2 cells compared with the growth of untreated control cells, as concluded from their low IC_{50} value 36.14 μ*M*. However, compounds **8a** and **3b** represents a moderate antitumor agent against Hep-G2 cells. Figure 2 shows the effect of compounds **3a–c** and **8a,b** on the growth of Hep-G2 cells. As measured by MTT assay, results are represented as percentage of control untreated cells.

Antioxidant activity. DPPH is a stable nonphysiological, radical, which could provide a relative figure of the radical scavenging activity of the tested compounds. The DPPH assay showed that some of the tested compounds possessed no scavenging activity to DPPH with high SC_{50} values (>100 μ*M*) compared to the scavenging activity (SC_{50} 8.41) of the well-known antioxidant (ascorbic acid, A.A), except compounds **3b**, **3c**, and **8a** which

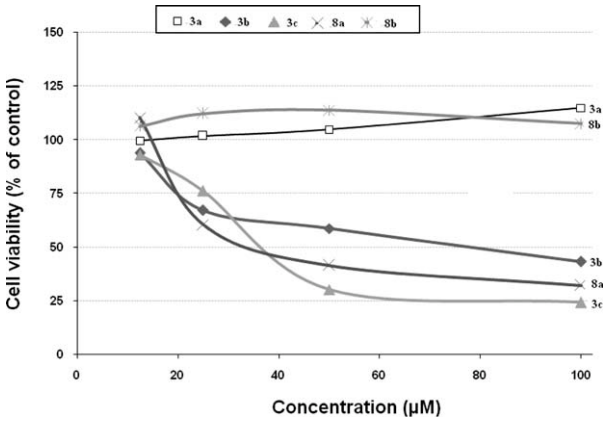


Figure 2. The effect of compounds **3a,c** and **8a,b** on the growth Hep-G2 cells. As measured by MTT assay. Results are represented as percentage of control untreated cells.

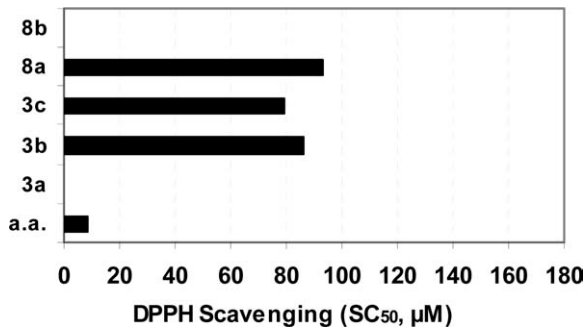


Figure 3. The antioxidant activity of **3b**, **3c**, and **8a** was investigated using DPPH assay. The results are represented as SC_{50} values (μM) as (mean ± SE, $n = 4$)

had effective antioxidant activity with SC_{50} values of 86.4, 79.2, and 92.8 μ*M*, respectively (Fig. 3).

EXPERIMENTAL

Chemistry. TLC analysis was performed on analytical Merck 9385 silica aluminium sheets (Kiselgel 60) with PF_{254} indicator. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. The IR spectra were recorded as KBr disks on Shimadzu-408 infrared spectrophotometer, Faculty of Science, El-Minia University. The NMR spectra were measured using Bruker AV-400, Florida Institute of Technology, USA. Chemical shifts were expressed as δ (ppm) with tetramethylsilane as internal reference. The samples were dissolved in chloroform-d_6 and/or dimethyl sulphoxide (DMSO)-d_6, s = singlet, d = doublet, dd = doublet of doublet, and t = triplet. Mass spectra were recorded on Varian MAT 312 instrument in E1 mode (70 eV), Technische Universität Braunschweig, Germany. Elemental analyses were performed using Varian Elementary device in National Research Center (Dokki, Giza, Egypt).

Materials. Dimethyl but-2-ynedioate (**2**) and thiocarbohydrazide (**6**) were bought from Fluka. Diaroyl thiocarbohydrazides **1a–c** were prepared according to the literature [26].

Reactions between diaroyl thiocarbohyhydrazides 1a–c with 2. An equal mixture of **1a–c** (1 mmol) and **2** (0.142 g, 1 mmol) was heated at reflux in absolute ethanol for 1–5 h (the reaction was followed by TLC analysis). The solvent was evaporated under vacuum and the obtained yellow precipitates were dissolved in dichloromethane and applied on column chromatography (dichloromethane, silica gel). The obtained products **3a–c** were recrystallized from the stated solvents.

*(Z)-Methyl-2-[(Z)-3-benzamido-2-(2-benzoylhydrazono)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**3a**).* Yellow crystals (toluene), yield = 323 mg (76%), m.p. 261–263°C. IR (potassium bromide): ν = 3240, (NH), 3070–3010 (Ar-CH), 2985–2875 (aliph.-CH), 1742, 1696, 1663 (C=O), 1618 (C=N) cm^{-1}. ^1H NMR (400.13 MHz, DMSO-d_6): δ_H = 11.78 (b, s, 1H, benzamido-NH), 11.44 (b, s, 1H, hydrazino-NH), 7.99 (d, 2H, H-2′, J = 7.5 Hz), 7.87 (d, 2H, H-2″, J = 7.0 Hz), 7.71 (t, 1H, H-4′, J = 7.4 Hz), 7.61 (t, 3H, H-3″,4″, J = 7.4, 7.7 Hz), 7.53 (t, 2H, H-3′, J = 7.2 Hz), 6.98 (s, 1H, vinylic-H), 3.81 (s, OCH_3) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C = 165.6 (2 benzoyl C=O), 164.5 (C-4), 160.8 (ester C=O), 151.9 (C-2), 137.4 (C-5), 132.8 (C-4′), 132.6 (C-1′), 131.7 (C-4″), 130.6 (C-1″), 128.7 (C-3′), 128.4 (C-3″), 127.7 (C-2′), 127.5 (C-2″), 116.9 (vinylic-CH), 52.7 (OCH_3) ppm. MS (70 eV, EI); *m/z* (%) = 424 [M+] (24), 312 (20), 283 (32), 281 (100), 138 (20), 104 (63), 91 (25), 77 (96), 69 (24), 57 (14), 51 (24). *Anal*. Calcd. for $C_{20}H_{16}N_4O_5S$ (424.43): C, 56.60; H, 3.80; N, 13.20; S, 7.55. Found: C, 56.50; H, 3.82; N, 13.28; S, 7.86.

*(Z)-Methyl-2-[(Z)-3-(4-methylbenzamido)-2-(2-(4-methylbenzoyl)-hydrazono)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**3b**).* Yellow crystals (methanol), yield = 353 mg (78%), m.p. 270–271°C. IR (potassium bromide): ν = 3070–3005 (Ar-CH), 2990–2850 (aliph.-CH), 1740, 1680, 1640 (C=O), 1612 (C=N) cm^{-1}. The NMR: Table 1. MS (70 eV, EI); *m/z* (%) = 452 [M^+] (24), 375 (18), 343.23 (30), 119 (100), 91 (27), 65 (30). *Anal*. Calcd. for $C_{22}H_{20}N_4O_5S$ (452.48): C, 58.40; H, 4.46; N, 12.38; S, 7.09. Found: C, 58.38; H, 4.63; N, 12.43; S, 7.23.

*(Z)-Methyl-2-[(Z)-3-(1-napthamido)-2-(2-(1-naphthoyl)hydrazono)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**3c**).* Yellow crystals (methanol), yield = 430 mg (82%), m.p. 259–260°C. IR (potassium bromide): ν = 3240, (NH), 3035–3005 (Ar-CH), 2985–2910 (aliph.-CH), 1740, 1691, 1670, 1953 (C=O), 1610 (C=N) cm^{-1}. ^1H NMR (400.13 MHz, DMSO-d_6): δ_H = 11.81 (b, s, 1H, napthamido-NH), 11.70 (b, s, 1H, hydrazino-NH), 8.55 (d, 1H, H-8′, J = 8.1 Hz), 8.22 (d, 1H, H-8″, J = 6.4 Hz), 8.17 (d, 1H, H-4′, J = 7.8 Hz), 8.12 (d, 1H, H-4″, J = 8.0 Hz), 8.06–7.95 (m, 2H, H-3′,3″), 7.89 (d, 1H, H-2′, J = 6.2 Hz), 7.78 (d, 1H, H-2″, J = 6.6 Hz), 7.69 (d, 2H, H-5′,5″, J = 7.4 Hz), 7.64–7.56 (m, 4H, H-6′,7″, 6″,7″), 7.02 (s, 1H, vinylic-H), 3.84 (s, OCH_3) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C = 166.9 (napthamido-C=O), 165.7 (ester-C=O), 165.1 (C=O), 160.6 (C-4), 147.8 (C-2), 137.6 (C-5), 133.2 (C-8a′), 133.1 (C-4a′), 132.3 (C-8a″), 131.2 (C-4′), 130.8 (C-1′), 130.5 (C-4″), 130 (C-3′), 129.8 (C-3″), 128.3 (C-4a″), 128.2 (C-1″), 127.4 (C-7′), 127.0 (C-7″), 126.6 (C-6′, C-6″), 126.4 (C-2′), 126.2 (C-2″), 126.1 (C-8′), 125.4 (C-8″), 124.9 (C-5′, C-5″), 116.8 (vinylic-CH), 52.7 (OCH_3) ppm. MS (FAB, 70 eV); *m/z* (%) = 524 [M^+] (100). *Anal*. Calcd. for $C_{28}H_{20}N_4O_5S$ (524.55): C, 64.11; H, 3.84; N, 10.68; S, 6.11. Found: C, 63.87; H, 3.95; N, 10.73; S, 6.21.

Synthesis of N-(2-propan-2-ylidene)-hydrazine-carbonothioyl)arylhydrazides 7a–c. To a suspension solution of **6** (0.106 g, 1 mmol) and $NaHCO_3$ (0.126 g, 1.5 mmol) in dry acetone (20 mL) was stirred at room temperature, the corresponding acid chloride (1 mmol) in dry acetone (5 mL) was added dropwise over a period of 20 min. The reaction mixture was stirred for further continued 3 h at room temperature then at refluxing temperature for 15 min. The reaction mixture was filtered and the salt precipitate was washed three times with chloroform (20 mL). The solvent of the filtrate was removed under vacuum. The obtained precipitate was then washed three times with 0.1*N* HCl (5 mL) followed by three times with water (30 mL). The obtained products **7a–c** were recrystallized from glacial acetic acid.

*4-Methyl-N-(2-propan-2-ylidene)hydrazinecarbono-thionyl)-benzamide (**7a**).* White crystals, yield = 225 mg (90%), m.p. 172–174°C. IR (potassium bromide): ν = 3230–3315 (NH), 3041–3009 (Ar-CH), 2981–2915 (aliph.-CH), 1674 (C=O), 1617 (C=N), 1365 (C=S) cm^{-1}. ^1H NMR (400.13 MHz, chloroform-d_3): δ_H = 9.98 (b, s, 1H, NH-2), 9.76 (b, s, 1H, NH-1), 9.24 (b, s, 1H, NH-3), 7.92 (d, 2H, H-2, J = 7.8 Hz),

7.57 (t, 1H, H-4, J = 7.5 Hz), 7.41 (t, 2H, H-3, J = 7.5 Hz), 1.97 (s, 3H, CH_3^a), 1.89 (s, 3H, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 132.8 (C-4), 131.5 (C-1), 128.6 (C-3), 128.4 (C-2), 24.8 (CH_3^a), 19.4 (CH_3^b) ppm. MS (70 eV, EI); m/z (%) = 250 [M^+] (40), 105 (100), 77 (42), 56 (31). *Anal*. Calcd. for $C_{11}H_{14}N_4OS$ (250.32): C, 52.78; H, 5.64; N, 22.38; S, 12.81. Found: C, 53.03; H, 5.50; N, 22.54; S, 12.97.

*4-Methyl-N-(2-(propan-2-ylidene)hydrazine-carbonothioyl)-benzohydrazide (**7b**)*. White crystals, yield = 243 mg (92%), m.p. 179–181°C. IR (potassium bromide): ν = 3234–3321 (NH), 3033–3005 (Ar-CH), 2978–2914 (aliph.-CH), 1679 (C=O), 1619 (C=N), 1359 (C=S) cm^{-1}. ^{1}H NMR (400.13 MHz, chloroform-d_3): δ_C = 9.96 (b, s, 1H, NH-2), 9.72 (b, s, 1H, NH-1), 9.44 (b, s, 1H, NH-3), 7.83 (d, 2H, H-2, J = 7.6 Hz), 7.23 (d, 2H, H-3, J = 7.6 Hz), 2.33 (3H, Ar-CH_3), 1.96 (s, 3H, CH_3^a), 1.90 (s, 3H, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 176.4 (C=S), 164.7 (benzoyl C=O), 156.5 (C(CH3)2), 143.4 (C-4), 132.8 (C-1), 129.4 (C-3), 127.6 (C-2), 24.9 (CH_3^a), 21.5 (Ar-CH_3), 19.6 (CH_3^b) ppm. MS (70 eV, EI); m/z (%) = 264 [M^+] (30), 119 (100), 91 (32), 56 (27). *Anal*. Calcd. for $C_{12}H_{16}N_4OS$ (264.35): C, 54.52; H, 6.10; N, 21.19; S, 12.13. Found: C, 54.63; H, 6.23; N, 21.41; S, 12.27.

*4-Methyl-N-(2-(propan-2-ylidene)hydrazine-carbono-thioyl)-naphthamide (**7c**)*. White crystals, yield = 288 mg (96%), m.p. 181–183°C. IR (potassium bromide): ν = 3242–3316 (NH), 3030–3012 (Ar-CH), 2981–2919 (aliph.-CH), 1669 (C=O), 1624 (C=N), 1341 (C=S) cm^{-1} ^{1}H NMR (400.13 MHz, chloroorm-d_3): δ_H = 10.7 (b, s, 1H, NH-3), 10 17 (b, s, 1H, NH-1), 10.11 (b, s, 1H, NH-2), 9.0 (d, 1H, H-8, J = 8.1 Hz), 8.05 (d, 1H, H-2, J = 7.4 Hz), 7.98 (d, 1H, H-4, J = 8.0 Hz), 7.91 (d, 1H, H-5, J = 8.0 Hz), 7.79 (dd, 1H, H-7, J = 7.7, 7.5 Hz), 7.55 (dd, 1H, H-H-6, J = 7.6, 7.4 Hz), 7.48 (t, 1H, H-3, J = 8.0 Hz), 1.98 (s, 3H, CH_3^a), 1.91 (s, 3H, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 76.4 (C=S), 165.3 (napthoyl C=O), 159.7 (N=C(CH_3)$_2$), 137.2 (C-4), 136.8 (C-4a), 133.5 (C-1), 132.4 (C-2), 131.6 (C-8a), 131.1 (C-3), 129.3 (C-5), 129.2 (C-7), 128.6 (C-8), 127.8 (C-6), 25.1 (CH_3^a), 19.9 (CH_3^b) ppm. MS (70 eV, EI); m/z (%) = 300 [M^+] (36), 155 (100), 127 (23), 56 (19). *Anal*. Calcd. for $C_{15}H_{16}N_4OS$ (300.38): C, 59.98; H, 5.37; N, 18.65; S, 10.67. Found: C, 59.73; H, 5.23; N, 18.41; S, 10.47.

Reactions between aroyl thiocarbohyhydrazides 7a–c with 2. As previously mentioned before: an equal mixture of **7a–c** (1 mmol) and 2 (0.142 g, 1 mmol) was heated at reflux in absolute ethanol for 10–14 h (the reaction was followed by TLC analysis). The solvent was evaporated under vacuum. The obtained products were then dissolved in dichloromethane and applied on column chromatography (dichloromethane, silica gel). The obtained pure products were recrystallized from the stated solvents.

*(Z)-Methyl-2-[(Z)-2-(2-benzoylhydrazono)-4-oxo-3-(propan-2-ylideneamino)-1,3-thiazolidin-5-ylidene]-acetate (**8a**)*. Yellow crystals (methanol), yield = 296 mg (82%), m.p. 216–218°C. IR (potassium bromide): ν = 3235 (NH), 3063–3015 (Ar-CH), 2995–2905 (aliph.-CH), 1736, 1698, 1672 (C=O), 1642, 1608 (C=N) cm^{-1}. ^{1}H NMR (400.13 MHz, chloroform-d_3): δ_H = 8.30 (b, s, 1H, hydrazino-NH), 7.91 (d, 2H, H-2′, J = 7.6 Hz), 7.60 (t, 1H, H-4′, J = 7.4 Hz), 7.49 (t, 2H, H-3′, J = 7.6 Hz), 6.96 (s, 1H, vinylic-H), 3.87 (s, OCH_3), 2.06 (s, CH_3^a), 1.94 (s, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 168.9 (C(CH3)2), 166.1 (ester C=O), 166 (benzoyl C=O), 162.1 (C-4), 152.5 (C-2), 139.4 (C-5), 133 (C-4′), 130.9 (C-1′), 128.9 (C-3′), 127.7 (C-2′), 117.3 (vinylic-CH), 52.6 (OCH_3), 25.0 (CH_3^a), 18.7 (CH_3^b) ppm. MS (70 eV, EI); m/z (%) = 360 [M^+] (30), 217 (28), 105 (100), 77 (32), 56 (20). *Anal*. Calcd. for $C_{16}H_{16}N_4O_4S$ (360.39): C, 53.32; H, 4.47; N, 15.55; S, 8.90. Found: C, 53.50; H, 4.50; N, 15.34; S, 8.97.

*(Z)-Methyl-2-[(Z)-2-(2-(4-methylbenzoyl)-hydrazono)-4-oxo-3-(propan-2-ylidene-amino)-1,3-thiazolidin-5-ylidene]-acetate (**8b**)*. Yellow crystals (methanol), yield = 315 mg (84%), m.p. 245–247°C. IR (potassium bromide): ν = 3200 (NH), 3070–3019 (Ar-CH), 2976–2873 (aliph.-CH), 1735, 1695, 1665 (C=O), 1645, 1607 (C=N) cm^{-1}. ^{1}H NMR (400.13 MHz, chloroform-d_3): δ_H = 8.45 (b, s, 1H, hydrazino-NH), 7.80 (d, 2H, H-2′, J = 8.0 Hz), 7.27 (d, 2H, H-3′, J = 7.9 Hz), 6.93 (s, 1H, vinylic-H), 3.86 (s, OCH_3), 2.41 (benzoyl CH_3), 2.04 (s, CH_3^a), 1.92 (s, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 168.9 (N=C(CH_3)$_2$), 166.1 (ester C=O), 165.3 (benzoyl C=O), 162.2 (C-4), 152.6 (C-2), 143.7 (C-4′), 139.5 (C-5), 129.5 (C-3′), 128.0 (C-1′), 127.7 (C-2′), 117.2 (vinylic-CH), 52.5 (OCH_3), 25.0 (CH_3^a), 21.6 (benzoyl CH_3) 18.7 (CH_3^b) ppm. MS (70 eV, EI); m/z (%) = 374 [M^+] (24), 275 (18), 119 (100), 91 (17), 56 (12). *Anal*. Calcd. for $C_{17}H_{18}N_4O_4S$ (374.41): C, 54.53; H, 4.85; N, 14.96; S, 8.56. Found: C, 54.24; H, 5.07; N, 14.82; S, 8.67.

*(Z)-Methyl-2-[(Z)-2-(2-(1-napthoyl)hydrazono)-4-oxo-3-(propan-2-ylideneamino)-1,3-thiazolidin-5-ylidene]-acetate (**8c**)*. Yellow crystals (methanol), yield = 361 mg (88%), m.p. 224–225°C. IR (potassium bromide): ν = 3064–3006 (Ar-CH), 2968–2879 (aliph.-CH), 1729, 1698, 1671 (C=O), 1648, 1612 (C=N) cm^{-1}. ^{1}H NMR (400.13 MHz, chloroform-d_3): δ_H = 9.60 (b, s, 1H, hydrazino-NH), 9.14 (d, 1H, H-8′, J = 8.4 Hz), 8.23 (d, 1H, H-2′, J = 7.2 Hz), 7.95 (d, 1H, H-4′, J = 8.0 Hz), 7.82 (d, 1H, H-5′, J = 8.1 Hz), 7.65 (dd, 1H, H-7′, J = 7.7, 7.5 Hz), 7.51 (dd, 1H, H-6′, J = 7.5, 7.3 Hz), 7.49 (t, 1H, H-3′, J = 7.9 Hz), 6.95 (s, 1H, vinylic-H), 3.94 (s, OCH_3), 2.07 (s, CH_3^a), 1.96 (s, CH_3^b) ppm. ^{13}C NMR (100.6 MHz, chloroform-d_3): δ_C = 169.3 (N=C(CH3)2), 167.4 (ester C=O), 167.2 (napthoyl C=O), 164.7 (C-4), 153.9 (C-2), 141.2 (C-4′), 140.3 (C-5), 134.6 (C-4′a), 133.2 (C-2′), 132.5 (C-8′^{a}), 131.3 (C-1′), 130.2 (C-5′), 129.6 (C-7′), 128.8 (C-8′), 128.1 (C-6′), 127.8 (C-3′), 118.3 (vinylic-CH), 52.8 (OCH_3), 25.5 (CH_3^a), 19.3 (CH_3^b) ppm. MS (70 eV, FAB); m/z (%) = 410 [M^+] (100). *Anal*. Calcd. for $C_{20}H_{18}N_4O_4S$ (410.45): C, 58.53; H, 4.42; N, 13.65; S, 7.81. Found: C, 58.28; H, 4.67; N, 13.82; S, 7.67.

Biological section.

Cell culture. Human hepatocellular carcinoma (HepG2) cells were routinely cultured in Dulbeco's Modified Eagle's Medium. Media were supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, containing 100 units/mL penicillin G sodium, 100 units/mL streptomycin sulphate, and 250 ng/mL amphotericin B. Cells were maintained at subconfluency at 37°C in humidified air containing 5% CO_2. For subculturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested samples were dissolved in DMSO. All cell culture material was obtained from Cambrex BioScience (Copenhagen,

Denmark). All chemicals were from Sigma/Aldrich, except mentioned. All experiments were repeated three times, unless mentioned.

Cytotoxicty assay. Cytotoxicity of tested samples was measured using the MTT cell viability assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm [31].

Reagents preparation. MTT solution: 5 mg/mL of MTT in 0.9% NaCl. Acidified isopropanol: 0.04*N* HCl in absolute isopropanol.

Procedure. Cells (0.5×10^5 cells/well) in serum-free media were placed in a flat bottom 96-well microplate and treated with 20 μL of different concentrations of each tested compound for 20 h at 37°C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 μL MTT solution/well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 μL of acidified isopropanol/well and plate was shacked at room temperature, followed by the photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated.

Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability.

Calculations. Percentage of relative viability was calculated using the following equation: [Absorbance of treated cells/Absorbance of control cells)] × 100.

Then the half maximal inhibitory concentration IC50 was calculated from the equation of the dose response curve.

Antioxidant activity (scavenging of DPPH). 1,1-Diphenyl-2-picrylhydrazyl is a stable deep violet radical due to its unpaired electron. In the presence of an antioxidant radical scavenger, which can donate an electron to DPPH, the deep violet color decolorize to the pale yellow nonradical form [32]. The change in colorization and the subsequent fall in absorbance are monitored spectrophotometrically at $\nu = 520$ nm.

Reagents preparation. Ethanolic DPPH: 0.1 m*M* DPPH/absolute ethanol, standard ascorbic acid solution. Serial dilutions of ascorbic acid in concentrations ranging from 0–2.5 μ*M* in distilled water. A standard calibration curve was plotted using serial dilutions of ascorbic acid in concentrations ranging from 0–2.5 μ*M* in distilled water.

Procedure. In a flat bottom 96-well microplates, a total test volume of 200 μL was used. In each well, 20 μL of different concentrations (0–100 μg/mL final concentration) of tested compounds were mixed with 180 μL of ethanolic DPPH and incubated for 30 min at 37°C. Triplicate wells were prepared for each concentration and the average was calculated. Then, the photometric determination of absorbance at $\nu = 515$ nm was done using microplate ELISA reader.

Calculations. The half-maximal scavenging capacity (SC_{50}) values for each tested compounds and ascorbic acid was estimated via two competitive dose curves.

Abs50 of ascorbic acid = (Abs100 − Abs0)/2.

SC_{50} of ascorbic acid was calculated using the curve equation.

SC_{50} of each compound was determined using the curve equation using Abs50 of ascorbic acid.

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