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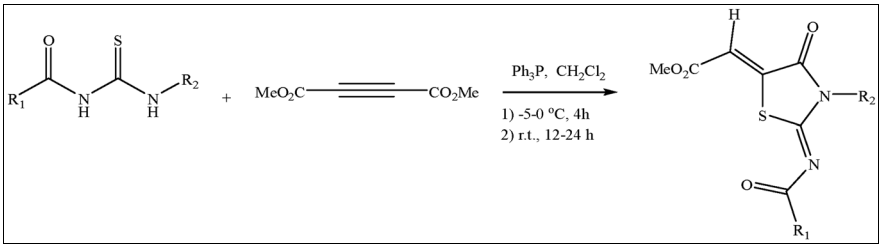


Reaction of 3-aroyl-1-arylthioureas with dimethyl but-2-ynedioate in dichloromethane and catalyzed by triphenylphosphine at −5°C led to (*Z*)-methyl 2-[(*Z*)-2-(4-aroylimino)-4-oxo-3-aryl-1,3-thiazolidin-5-ylidene] acetates in good yields. The mechanism is discussed. X-ray structure analysis of one thiazolidine derivative is described. Antitumor and antioxidant activities have been investigated. One derivative of 1,3-thiazolidine showed moderate antiproliferative *in vitro* activity against hepatocellular carcinoma Hep-G2, whereas another 1,3-thiazolidine introduced effective antioxidant activity compared to ascorbic acid.

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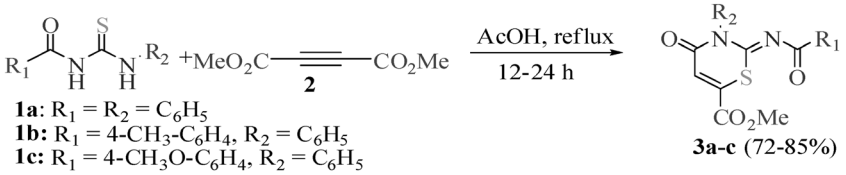
## INTRODUCTION

Thiazolidin-4-one ring systems are known to act as analgesic, antibacterial, anticonvulsant, antiparasitic, anti-inflammatory, herbicidal agents [2–7], and potent anti-HIV agents [8]. In view of the biological activities of thiazolidinones, several approaches for their synthesis have been described [9]. Imidazolidine-2-thiones were obtained from the oxidative cyclization of 1-benzoyl-3-aryl-thioureas with bromine and enolizable carbonyl compounds in the presence of excess triethylamine [10,11]. Manaka [12] reported the one-pot condensation of aroyl-arylthiourea with α-halocarbonyl derivatives to afford 2-acylimino-3-alkyl-3*H*-thiazolines. Aly *et al.* have demonstrated a convenient synthesis of fused thiazoles from the reaction of aroylphenyl thioureas with π-acceptor quinones [13], but the reactions of *N*-aroyl-*N*′-arylthioureas with 2,3-diphenyl-cyclopropenone afford *E*/*Z* mixtures of 3-(3′-aroylthioureido)-2,3-diphenyl-cinnamicacids [14]. It is also known that the reactions of amidinothioureas, imidoylthioureas, and thioacylamidines with diethyl azodicarboxylate give the corresponding thiadiazoles by oxidative cyclic *S*-*N* bond formation [15]. 1-Acylthiosemicarbazides, react with phenyl propiolate in acetic acid under reflux to afford triazolothiazines [16]. Aly *et al.* recently demonstrated that the reaction of *N*-aroyl-*N*′-arylthioureas 1a-c with dimethyl but-2-ynedioate (2) under reflux in acetic acid yields the corresponding 1,3-thiazinones 3a-c (Scheme 1) [17].

## RESULTS AND DISCUSSION

**Chemistry.** The protocol (Scheme 2) was as follows. A solution of **2** was mixed together with triphenylphosphine at −5–0°C for 4 h. *N*-Aroyl-*N*′-arylthiourea **1a-f** was then added at 0°C, and the reaction mixture was stirred at room temperature for 12–24 h. TLC indicated that the products differ in $R_f$ from authentic samples [17] of thiazin-4-ones **3a-c**. The isolated products were identified as thiazolidin-4-ones **4a-f** by NMR, IR, and mass spectra along with elemental analyses (Scheme 2). Figure 1 shows numbering of compounds **4d** (exemplifying **4a-e**) and **4f**. The $^{13}$C-NMR shifts in the new ring are similar to each other throughout the series (Table 1), and are quite different from those of **3a-c** [17]. In $^{1}$H-coupled $^{13}$C-NMR spectra of **4c**, **4d**, and **4f**, the lactam carbonyls C-4 appear as doublets, whose coupling constants (5.8–5.9 Hz) require that the couplings span three bonds and that C-4

**Scheme 1.** Synthesis of 1,3-thiazin-4-ones **3a-c**.




**1a**: $R_1 = R_2 = C_6H_5$
**1b**: $R_1 = 4\text{-}CH_3\text{-}C_6H_4$, $R_2 = C_6H_5$
**1c**: $R_1 = 4\text{-}CH_3O\text{-}C_6H_4$, $R_2 = C_6H_5$

**3a-c** (72-85%)

and the vinylic H be mutually *cis* [18–20]. The structure of **4d** was unambiguously proved by X-ray structure analysis (Fig. 2). The mechanism depends upon the known nucleophilic character of triphenylphosphine, which catalyzes the conjugate addition of the sulfur lone pair to the acetylenic ester [21].

Crystals of general formula, $C_{20}H_{15}IN_2O_4S$, were obtained by slow evaporation of dichloromethane, Mr 506.31, orthorhombic Pbca, $Z = 8$, $a = 15.2205(3)$ Å, $b = 10.7102(2)$ Å, $c = 24.6618(5)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 4020.23(14)$ Å$^3$, $D_x = 1.673$ Mg m$^{-3}$, $\theta = 2.910–22.986°$, $\mu = 1.73$ mm$^{-1}$, *M*o ($\lambda = 0.71073$ Å), $T = 298$ K. The crystallographic data of **4d** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 705627.

**Biological investigation.** *Cytotoxicity of compounds 4a-f against Hep-G2 cells.* It was found that **4f** showed moderate antiproliferative *in vitro* activity against hepatocellular carcinoma Hep-G2, Using the MTT Cell Viability Assay [22], we studied the effect of compounds **4d-f** 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 μM). Figure 3 indicates the effect of compounds **4d-f** on the growth of Hep-G2 cells.

*Antioxidant activity.* 1,1-Diphenyl-2-picrylhydrazyl (DPPH) 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 decolourizes to the pale yellow nonradical form [23]. The DPPH assay showed that both **4d** and **4f** possessed no scavenging activity to DPPH with high $SC_{50}$ values (>100 μM) compared to the scavenging activity ($SC_{50} = 8.41$ μM) of the well-known antioxidant ascorbic acid. On the other hand compound **4e** has effective antioxidant activity, with an $SC_{50}$ value of 99.3 μM.

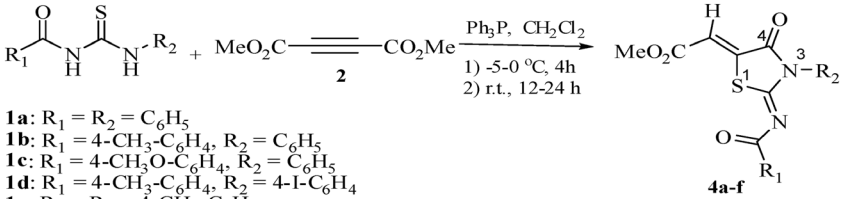
## EXPERIMENTAL

**Chemistry.** TLC was performed on analytical Merck 9385 silica aluminium sheets (Kieselgel 60) with $PF_{254}$ indicator. TLCs were viewed under ν = 254 nm. Column chromatography (CC) was packed with silica gel. Melting points were determined on a Stuart electrothermal melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Shimadzu-408 infrared spectrophotometer, Faculty of Science, Minia University. NMR spectra were measured in $CDCl_3$, at 400 MHz for $^1$H and 100 MHz for $^{13}$C, using a Bruker AV-400 spectrometer at Florida Institute of Technology. Chemical shifts are expressed as δ (ppm) *vs.* tetramethylsilane (TMS) = 0. Coupling constants are stated in Hz; $^1$H-coupled $^{13}$C spectra were measured using gated decoupling. Correlations were established using $^1$H-$^1$H COSY, HMQC, HSQC, and HMBC experiments. Mass spectral data were recorded on Varian MAT 312 instrument in EI mode (70 eV) at the Technische Universität Braunschweig, Germany. Elemental analyses were carried out using Varian El Elementar in National Research Center, Giza, Egypt. X ray analysis was carried on Bruker Nonius, Delft & MacScience, National Research Center, Giza, Egypt.

***Materials.*** Dimethyl but-2-ynedioate ethyl ester (DMAD, **2**) was bought from Fluka. *N*-Aroyl-*N*′-arylthioureas **1a-f** were prepared according to the literature [24].

*N-(4-Iodophenyl)-N′-(4-methylbenzoyl)thiourea (**1d**).* White crystals (EtOH) 333 mg (84%), m.p. 195–197°C. IR: ν = 3310, 3340 (2NH), 3090–3009 (Ar—CH), 2960–2830 (aliph. —CH), 1720 (C=O) cm$^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 12.66 (bs, 1H, N′H), 9.05 (bs, 1H, NH), 7.78 (d, *J* = 8.2, 2H; H-2′), 7.73 (d, *J* = 8.5, 2H; H-3), 7.52 (d, *J* = 8.6, 2H; H-2), 7.35 (d, *J* = 8.0, 2H; H-3′), 2.46 (s, 3H; $CH_3$). $^{13}$C-NMR $CDCl_3$, 100 MHz): δ 178.4 (C=S),

**Scheme 2.** Reactions of *N*-aroyl-*N*′ -arylthioureas with DMAD; synthesis of new 1,3-thiazolidin-4-ones **4a-f**. Yields of **4a** (82%), **4b** (83%), **4c** (85%), **4d** (74%), **4e** (84%), **4f** (80%).




**1a**: $R_1 = R_2 = C_6H_5$
**1b**: $R_1 = 4\text{-}CH_3\text{-}C_6H_4$, $R_2 = C_6H_5$
**1c**: $R_1 = 4\text{-}CH_3O\text{-}C_6H_4$, $R_2 = C_6H_5$
**1d**: $R_1 = 4\text{-}CH_3\text{-}C_6H_4$, $R_2 = 4\text{-}I\text{-}C_6H_4$
**1e**: $R_1 = R_2 = 4\text{-}CH_3\text{-}C_6H_4$
**1f**: $R_1$ = 1-naphthyl, $R_2 = 4\text{-}CH_3O\text{-}C_6H_4$

**4a-f**

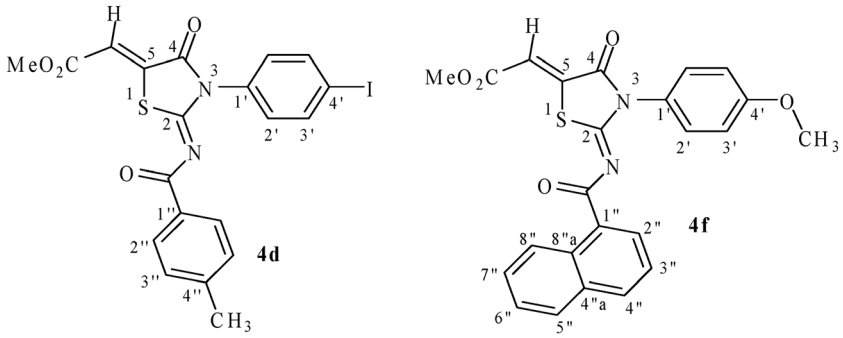



**Figure 1.** Numbering of thiazolidine derivatives **4d–f**.

166.9 (C=O), 145.1 (C-4′), 138.0 (C-1), 137.4 (C-3), 130.0 (C-3′), 128.5 (C-1′), 127.6 (C-2′), 125.7 (C-2), 91.1 (C-4), 21.7 ($CH_3$). MS; *m/z* (70 eV, %) = 396 (24), 395 [$M^+$] (28), 390 (22), 305 (24), 303 (44), 302 (60), 230 (14), 229 (18), 128 (42), 127 (100), 92 (38), 91 (40). Anal. Calcd. for $C_{15}H_{13}IN_2OS$ (396.25): C, 45.47; H, 3.31; N, 7.07; S, 8.09. Found: C, 45.66; H, 3.49; N, 6.88; S, 8.22.

***Reactions between N-aroyl-N′-aryl-thioureas and dimethyl but-2-ynedioate (2).*** To an ice cooled (−5°C), stirred suspension of triphenylphosphine (1 mmol) in absolute dry dichloromethane (10 mL) was added over 15 min a solution of **2** (1 mmol) in the same solvent (10 mL). At 0°C, a solution of aroyl-arylthioureas (**1**, 1 mmol) in absolute dichloromethane (25 mL) was added over 4 h. The reaction mixture was then stirred at room temperature for 12–24 h (the reaction was followed by TLC). The solvent was evaporated under vacuum and the residue was applied to a small column packed (silica gel) using dichloromethane as eluent.

*(Z)-Methyl-2-[(Z)-2-(benzoylimino)-4-oxo-3-phenyl-1,3-thiazolidine-5-ylidene]acetate (**4a**).* Yellowish white crystals (EtOAc), 325 mg (88.7%), m.p. 222–224°C (lit. [25], 223°C). IR: ν = 3080–2990 (Ar—CH), 1715, 1705, 1655 (C=O), 1605 (C=N), 1590 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 8.04–7.36 (m, 10H; aromatic H), 7.09 (s, 1H; vinylic H), 3.92 (s, 3H; $OCH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 172.6 (benzoyl C=O), 167.0 (C-4), 165.8 (ester C=O), 158.0 (C-2), 139.0 (C-4″), 138.6 (C-5), 134.2 (C-1″), 130.0 (C-1′), 128.2 (C-2″), 128.0 (C-3″), 127.6 (C-4″), 126.8 (C-4′), 125.8 (C-3′), 125.0 (C–2′), 119.0 (vinylic—CH=), 50.2 ($OCH_3$). MS; *m/z* (70 eV, %) = 366 [$M^+$] (38), 334 (40), 307 (18), 289 (28), 261 (24), 145 (14), 124 (29), 105 (100), 77 (37), 59 (17), 31 (22). Anal. Calcd. ($C_{19}H_{14}N_2O_4S$): C, 62.28; H, 3.85; N, 7.65. Found: C, 62.05; H, 3.83; N, 7.67.

*(Z)-Methyl-2-[(Z)-2-(4-methylbenzoylimino)-4-oxo-3-phenyl-1,3-thiazolidin-5-ylidene]acetate (**4b**).* Yellowish white crystals (EtOAc), 310 mg (81.5%), m.p. 236–237. IR: ν = 3097–3080 (Ar—CH), 2990–2870 (aliph.—CH), 1711, 1700, 1645 (C=O), 1602 (C=N), 1596 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 7.80 (d, *J* = 6.6, 2H; H-2″), 7.70–7.50 (m, 5H; aromatic H), 7.25 (d, *J* = 6.6, 2H; H-3″), 6.93 (s, 1H; vinylic-H), 3.85 (s, 3H; $OCH_3$), 2.34 (s, 3H, Ar-$CH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 173.4 (benzoyl—CO), 167.6 (C-4), 165.5 (ester—CO), 158.2 (C-2), 149.2 (C-5), 138.0 (C-4″), 137.8 (C-2″), 135.4 (C-1″), 130.4 (C-3″), 128.2 (C-4′), 127.6 (C-3′), 127.2 (C-1′), 126.8 (C-2′), 119.5 (vinylic-CH), 50.4 ($OCH_3$), 32.8 (Ar—$CH_3$). MS; *m/z* (70 eV, %) = 380 [$M^+$] (20), 265 (30), 349 (24), 303 (12), 124 (21), 119 (100), 91 (42), 77 (32), 59 (19), 31 (23). Anal. Calcd. ($C_{20}H_{16}N_2O_4S$) C, 63.14; H, 4.24; N, 7.36. Found: C, 63.05; H, 4.20; N, 7.24.

*(Z)-Methyl-2-[(Z)-2-(4-methoxybenzoylimino)-4-oxo-3-phenyl-1,3-thiazolidin-5-ylidene]-acetate (**4c**).* Yellow crystals (EtOAc), 325 mg (82%), m.p. 196–198°C. IR: ν = 3080–2980 (Ar—CH), 2980–2870 (aliph.—CH), 1731, 1707, 1665 (C=O), 1613 (C=N), 1587 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 7.98 (d, *J* = 8.9, 2H; H-2″), 7.59 (t, *J* = 7.3, 2H; H-3′), 7.55 (t, *J* =7.1, 1H; H-4′), 7.40 (d, *J* = 7.1, 2H; H-2′), 7.08 (s, 1H; vinylic-H), 6.86 (d, *J* = 9.0, 2H Hz; H-3″), 3.91 (s, 3H; $CO_2CH_3$), 3.84 (s, 3H; $ArOCH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 176.2 (t, *J* = 3.7; benzoyl—CO), 165.5 (dq, $J_d$ = 1.6, $J_q$ = 4.0; C-4), 165.1 (s; ester—C=O), 165.0 (d, *J* = 5.8 Hz; C-4), 164.1 (m; C-4″), 141.1 (s; C-5), 143.2 (tt, *J* = 9.5, 2.9; C-1′), 132.7 (dd, *J* = 163.2, 6.9; C-2″), 129.4 (dt, $J_d$ = 160.7, $J_t$ = 8.0; C-4′), 129.3 (dd, *J* = 163.7, 7.8; C-3′), 128.6 (ddd, *J* = 164.0, 6.7, 6.7; C-1′), 127.5 (t, *J* = 7.3; C-1″), 120.3 (d, *J* = 172.4; vinylic-CH=), 113.7 (dd, *J* = 161.2, 4.7; C-3″), 55.5 (q, *J* = 144.5; $ArOCH_3$), 52.8 (q, *J* = 147.9; $CO_2CH_3$). MS; *m/z* (70 eV, %) = 396 (34, $M^+$), 365 (21), 319 (18), 262 (23), 135 (100), 124 (19), 107 (13), 91 (24), 77 (27), 59 (20), 31 (18). Anal. Calcd. ($C_{20}H_{16}N_2O_5S$): C, 60.60; H, 4.07; N, 7.07. Found: C, 60.31; H, 4.56; N, 6.84.

**Table 1**

$^{13}$C-NMR shifts in the new rings of **4a-f** and **3a-c**.

| Compound | C2 | C4 | C5 | =CH | Compound | C2 | C4 | C6 | =CH |
|---|---|---|---|---|---|---|---|---|---|
| **4a** | 158.0 | 167.0 | 138.6 | 119.0 | **3a** [16] | 158.0 | 172.6 | 155.0 | 134.2 |
| **4b** | 158.2 | 167.6 | 149.2 | 119.5 | **3b** [16] | 158.2 | 173.4 | 155.2 | 135.4 |
| **4c** | 165.0 | 165.5 | 141.1 | 120.3 | **3c** [16] | 158.4 | 171.4 | 155.6 | 133.8 |
| **4d** | 164.7 | 165.4 | 140.7 | 120.8 | | | | | |
| **4e** | 159.6 | 164.7 | 141.1 | 121.0 | | | | | |
| **4f** | 165.2 | 165.7 | 141.2 | 120.3 | | | | | |

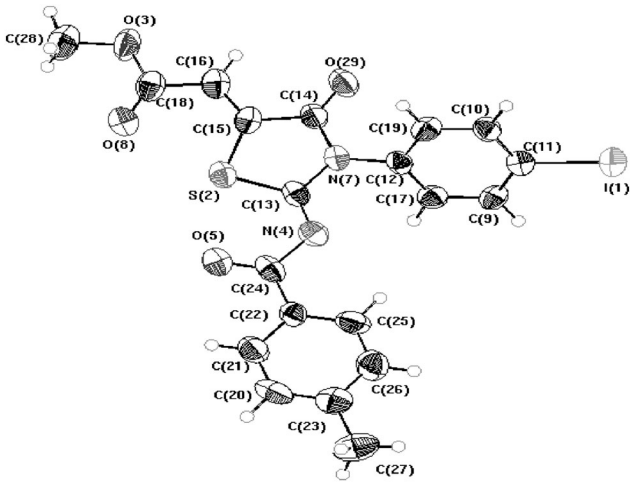


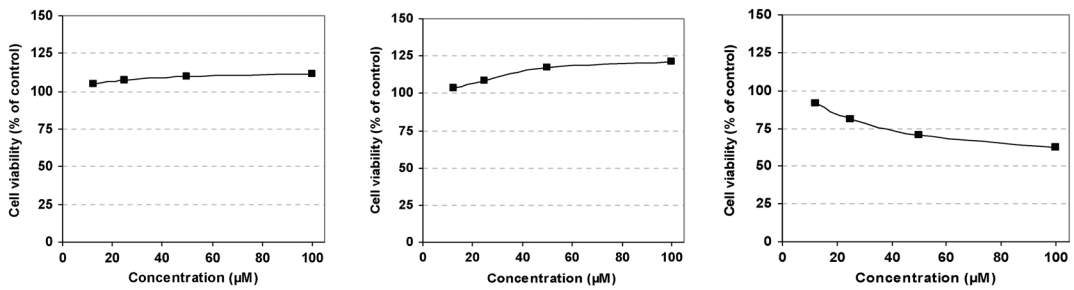

**Figure 2.** X-ray structure analysis of compound **4d**.

*(Z)-Methyl-2-[(Z)-3-(4-iodophenyl)-2-(4-methylbenzoylimino)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**4d**).* Pale yellow crystals (ethyl acetate), 410 mg (81%), m.p. 239–240°C. IR: ν = 3080–3009 (Ar—CH), 2986–2850 (aliph.—CH), 1722, 1705, 1645 (C=O), 1610 (C=N), 1538 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 7.91 (d, *J* = 8.4, 4H; H-2′,2″), 7.21 (d, *J* = 8.0, 2H; H-3″), 7.17 (d, *J* = 8.6, 2H; H-3′), 7.06 (s, 1H; vinylic-H), 3.90 (s, 3H; $CO_2CH_3$), 2.40 (s, 3H; $ArCH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 176.8 (t, *J* = 4.1; benzoyl—CO), 165.4 (dq, $J_d$ = 1.2, $J_q$ = 3.6; C-4), 165.3 (s; ester—C=O), 164.7 (d, *J* = 5.9; C-2), 144.8 (q, *J* = 6.9; C-4″), 140.7 (s; C-5), 138.5 (dd, *J* = 168.5, 6.6; C-2′), 133.7 (tt, *J* = 9.7, 2.6; C-1′), 131.9 (t, *J* = 7.5; C-1″), 130.5 (dd, *J* = 162.5, 6.1; C-2″), 129.5 (dd, *J* = 165.5, 5.9; C-3′), 129.3 (ddq, $J_d$ = 159.2, 5.2, $J_q$ = 5.2; C-3″), 120.8 (d, *J* = 172.6; vinylic—CH), 95.2 (tt, *J* = 10.4, 2.6; C-4′), 52.8 (q, *J* = 147.9; $OCH_3$), 30.9 (tq, $J_t$ = 4.4, $J_q$ = 127.0; $ArCH_3$). MS: *m/z* (70 eV, %) = 508 [M+2], (15), 506 [$M^+$] (40), 490 (17), 474 (23), 447 (32), 415 (29), 379 (42), 245 (25), 174 (14), 124 (32), 119 (100), 91 (30), 65 (11), 59 (30), 43 (11), 31 (17). Anal. Calcd. ($C_{20}H_{15}IN_2O_4S$): C, 47.44; H, 2.99; N, 5.53. Found: C, 47.51; H, 2.94; N, 5.47.

*(Z)-Methyl-2-[(Z)-2-(4-methylbenzoylimino)-3-(4-methylphenyl)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**4e**).* Cottony white crystals (EtOAc), 335 mg (85%), m.p. 240–241°C. IR: ν = 3086–2995 (Ar—CH), 2890–2860 (aliph.—CH), 1722, 1705, 1647 (C=O), 1610 (C=N), 1530 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 7.93 (d, *J* =7.7, 2H; H-2″), 7.42 (d, 2H, *J* = 7.9 Hz; H-2′), 7.34 (d, 2H, *J* = 7.7 Hz; H-3″), 7.24 (d, 2H, *J* = 7.9 Hz; H-3′), 7.09 (s, 1H; vinylic-H), 3.92 (s, 3H; $CO_2CH_3$), 2.34 (s, 3H; Aroyl-$CH_3$), 2.32 (s, 3H; Ar-$CH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 176.3 (benzoyl-CO), 164.7 (C-4), 163.8 (ester-CO), 159.6 (C-2), 141.1 (C-5), 140.2 (C-4″), 139.8 (C-4′), 135.2 (C-1″), 132.4 (C-1′), 130.2 (C-3′), 129.6 (C-2′), 129.2 (C-2″), 128.5 (C-3″), 121.0 (vinylic-CH), 51.4 (ester-$CH_3$), 30.8 (Aroyl-$CH_3$), 28.7 (Ar-$CH_3$). MS; *m/z* (70 eV, %) = 394 (69, $M^+$), 379 (13), 363 (18), 275 (19), 124 (25), 105 (11), 91 (100), 59 (14), 31 (19). Anal. Calcd. ($C_{21}H_{18}N_2O_4S$): C, 63.94; H, 4.60; N, 7.10. Found: C, 63.59; H, 5.04; N, 7.15.

*(Z)-Methyl-2-[(Z)-3-(4-methoxyphenyl)-2-(1-napthoylimino)-4-oxo-1,3-thiazolidin-5-ylidene]-acetate (**4f**).* Canary yellow crystals (EtOAc), 390 mg (87%), m.p. 170–171°C. IR: ν = 3080–2960 (Ar—CH), 2980–2870 (aliph.—CH), 1720, 1705, 1650 (C=O), 1615 (C=N), 1595 (C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$, 400 MHz): δ = 9.26 (d, *J* = 8.6, 1H; H-8″), 8.30 (d, *J* = 7.1, 1H; H-2″), 8.01 (d, *J* = 8.1, 1H; H-4″), 7.86 (d, *J* = 8.0, 1H; H-5″), 7.61 (dd, *J* = 7.4, 7.2, 1H; H-7″), 7.53 (dd, *J* = 7.3, 7.2, 1H; H-6″), 7.41 (dd, *J* = 7.8, 7.8, 1H; H-3″), 7.34 (d, *J* = 8.9, 2H; H-2′), 7.089 (s, 1H; vinylic-H), 7.086 (d, *J* = 8.9, 2H; H-3′), 3.92 (s, 3H, $CO_2CH_3$), 3.90 (s, 3H; $ArOCH_3$). $^{13}$C-NMR: ($CDCl_3$, 100 MHz): δ = 178.4 (d, *J* = 5.2; naphthoyl-CO), 165.7 (dq, $J_d$ = 1.5, $J_q$ = 3.7; C-4), 165.2 (d, *J* = 5.8; C-2), 165.1 (s; ester—C=O), 160.1 (b; C-4′), 141.2 (s; C-5), 134.8 (ddd, *J* = 160.0, 6.5, 6.5; C-4″), 133.9 (ddd, *J* = 6.5, 6.5, 6.5; C-4″a), 132.9 (ddd, *J* = 163.2, 8.3, 2.0; C-2″), 131.9 (bdd, *J* = 7.4, 7.4; C-8″a), 130.5 (m; C-1″), 128.9 (dd, *J* = 162.0, 6.1; C-2′), 128.6 (ddd, *J* = 159.1, 6.1, 6.1; C-5″), 128.3 (dd, *J* = 160.3, 8.3; C-7″), 126.7 (tt, *J* = 9.9, 2.9; C-1′), 126.4 (dd, *J* = 166.2, 7.8; C-8″/6″), 126.3 (dd, *J* = 160.1, 9.0; C-6″/8″), 124.6 (d, *J* = 162.4; C-3″), 120.3 (d, *J* = 172.6; vinylic-CH), 114.6 (dd, *J* = 161.0, 5.1; C-3′), 55.6 (q, *J* = 144.2; Ar-$OCH_3$), 52.8 (q, *J* = 147.9; ester-$CH_3$). MS *m/z* (70 eV, %) = 446 (27, $M^+$), 415 (48), 387 (21), 319 (18), 291 (12), 155 (42), 127 (100), 124 (31), 59 (48), 31 (14). Anal. Calcd. ($C_{24}H_{18}N_2O_5S$): C, 64.56; H, 4.06; N, 6.27. Found: C, 64.69; H, 4.28; N, 6.35.

**Biology.** ***Cell culture.*** Hepatocellular carcinoma (HepG2) cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium). Media were supplemented with 10% fetal bovine serum (FBS), 2 mM 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




(a) (b) (c)

**Figure 3.** The effect of (a) **4d**, (b) **4e**, and (c) **4f** on the growth of Hep-G2 cells, as measured by MTT assay. Results are represented as percentage of control untreated cells.

were dissolved in dimethyl sulphoxide (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.

*Cytotoxicity assay.* Cytotoxicity of tested samples against Hepatocellular carcinoma (HepG2) 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 dark blue insoluble formazan crystals to which cell membranes are largely impermeable, 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 [22].

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

*Procedure.* Cells (0.5 X 105 cells/well) in serum-free media were plated 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 the plate was shaken at room temperature, followed by photometric determination of the absorbance at ν = 570 nm using a 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)] X 100. Then the half maximal inhibitory concentration $IC_{50}$ 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 decolorizes to the pale yellow nonradical form [23]. The change in colorization and the subsequent fall in absorbance are monitored spectrophotometrically at ν = 520 nm.

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

*Procedure.* In a flat bottom 96-well microplate, 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 were mixed and incubated for 30 min at 37°C. Triplicate wells were prepared for each concentration and the average was calculated. Then photometric determination of absorbance at ν = 515 nm was made, 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. $Abs_{50}$ of ascorbic acid = $(Abs_{100}-Abs_0)/2$. The $SC_{50}$ of ascorbic acid was calculated using the curve equation. $SC_{50}$ of each compound was determined using the curve equation utilizing $Abs_{50}$ of ascorbic acid.

## CONCLUSION

In conclusion, we have prepared novel thiazolidin-4-one derivatives *via* one-pot reaction between 3-aroyl-1-arylcarbonylthioureas with dimethyl but-2-ynedioate. The present method carries the advantage that the reaction is performed under neutral conditions. Since in general thiazolidines have shown antitumor activity, it is valuable to continue that investigation with the newly synthesized thiazolidines **4a**-**e**.

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### REFERENCES AND NOTES

[1] Current address: Chemistry Department, College of Science, Al-Jouf University, Sakaka, Al-Jouf, Saudi Arabia

[2] Li, Y. X.; Wang, S. H.; Li, Z. M.; Su, N.; Zhao, W. G. Carbohydrate Res 2006, 341, 2867.

[3] Kucukguzel, G.; Kocatepe, A.; De Clercq, E.; Sahin, F.; Gulluce, M. Eur J Med Chem 2006, 41, 353.

[4] Kato, T.; Ozaki, T.; Tamura, K.; Suzuki, Y.; Akima, M.; Ohi, N. J Med Chem 1998, 41, 4309.

[5] Tenorio, R. P.; Carvalho, C. S.; Pessanha, C. S.; de Lima, J. G.; de Faria, A. R.; Alves, A. J.; de Melo, E. J. T.; Goes, A. J. S. Bioorg Med Chem Lett 2005, 15, 2575.

[6] Kucukguzel, S. G.; Oruc, E. E.; Rollas, S.; Sahin, F.; Ozbek, A. Eur J Med Chem 2002, 37, 197.

[7] Shih, M. H.; Ke, F. Y. Bioorg Med Chem 2004, 12, 4633.

[8] Barreca, M. L.; Balzarini, J.; Chimirri, A.; De Clercq, E.; De Luca, L.; Holtje, H. D.; Holtje, M.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Rao, A.; Zappala, M. J Med Chem 2002, 45, 5410.

[9] Short, F. W.; Littleton, B. C.; Johnson, J. L. Chem Ind (London) 1971, 705.

[10] Hartung, J.; Rosenbaum, K.; Beyer, L.; Losada, J. Fernandez, V. J Prakt Chem 1991, 333, 537.

[11] Zeng, R.-S.; Zou, J.-P.; Zhi, S.-J.; Chen, J.; Shen, Q. Org Lett 2003, 5, 1657.

[12] Manaka, A.; Ishii, T.; Takahashi, K.; Sato, M. Tetrahedron Lett 2005, 46, 419.

[13] (a) Aly, A. A.; Ahmed, E. K.; El-Mokadem, K. M. J Sulf Chem 2006, 27, 419; (b) For a recent review on aroyl substituted thioureas and their applications, see: Aly, A. A.; Ahmed, E. K.; El-Mokadem, K. M. Hegazy, M. El-A. ibid 2007, 28, 73.

[14] Aly, A. A.; Ahmed, E. K.; El-Mokadem, K. M. J Sulf Chem 2007, 28, 285.

[15] Kihara, Y.; Kabashima, S.; Uno, K.; Okawara, T.; Yamasaki, T.; Furukawa, M. Synthesis 1990, 1020.

[16] Danilkina, N. A.; Mikhailov, L. E.; Ivin, B. A. In the 3rd Euro-Asian Heterocyclic Meeting "Heterocycles in organic and combinatorial chemistry" (EAHM-2004) September 12–17, 2004 Novosibirsk, Russia.

[17] Aly, A. A.; Ahmed, E. K.; El-Mokadem, K. M. J Heterocycl Chem 2007, 44, 1431.

[18] Nagase, H. Chem Pharm Bull 1973, 21, 270.

[19] Vögeli, U.; von Philipsborn, W.; Nagarajan, K.; Nair, M. D. Helv Chim Acta 1978, 61, 607.

[20] Danilkina, N. A.; Mikhailov, L. E.; Ivin, B. A. Russ J Org Chem 2006, 42, 783.

[21] Baharfar, R.; Baghbanian, S. M.; Hossein, N.; Bijanzadeh, H. R. Lett Org Chem 2007, 4, 567.

[22] Hansen, M. B.; Nielsen, S. E.; Berg, K. J Immunol Methods 1989, 119, 203.

[23] Van Amsterdam, F. T.; Roveri, A.; Maiorino, M.; Ratti, E.; Ursini, F. Free Rad Biol Med 1992, 12, 183.

[24] Hritzova, O.; Koscik, D. Coll Czech Chem Commun 1991, 56, 880.

[25] Marwaha, A.; Sudan, V.; Mahajan, M. P. Heterocycles 2008, 76, 1087.