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Synthesis, Single Crystal X-Ray, and Anticancer Activity of Some New Thiophene and 1,3-Thiazolidine Derivatives[1]

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Abstract—New series of thiophenes **6a–6e** and 1,3-thiazolidines **13a–13f** and **15a–15e** were synthesized starting from 2-(4-methoxybenzoyl)-3-(phenylamino)-3-thioxopropanoate **3**. The reaction of **3** with 1-aryl-2-bromoethanones **4a–4e** yielded thiophenes **6a–6e**. The X-ray single crystal analysis data accumulated for **6c** and its structural features can be extended to the analogues **6a, 6b** and **6d, 6e**. Treatment of **3** with ethyl 2-bromoacetate afforded 1,3-thiazolidinone **11** which upon treatment with aldehydes **12a-f** or isatins **14a–4e** gave 5-arylidene derivatives **13a–13f** and 4-oxo-5(-2-oxoindolin)-3-ylidenes **15a–15e**, respectively. The newly synthesized compounds were tested *in vitro* for their anti-cancer activity against two cell lines (HepG-2 and MCF-7) using MTT assay. Most of these compounds demonstrated a significant anticancer activity compared with that of doxorubicin. Isatin derivative **15a** was the most potent compound against HepG-2 cancer cells whereas *p*-MeO substituted benzylidine **13b** showed the highest anticancer activity against MCF-7 cancer cells. The fluoro substituted isatin **15d** showed anticancer activity more potent than doxorubicin against both HepG-2 and MCF-7 cancer cells, respectively.

Keywords thiophene, 1,3-thiazolidine, isatin, single crystal X-ray diffraction, anticancer

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INTRODUCTION

Thiophene-based derivatives have shown numerous biological activities such as analgesic [1], anti-inflammatory [2], antifungal [3] and antibacterial activities [4]. Thiophene-based compounds showed a promising antitumor activity [5–6]. For example, a series of functionalized thiophenes with general structure **1** [7] (Fig. 1) exhibited antiproliferative effect against human cells of cervical adenocarcinoma (HeLa), human pancreatic adenocarcinoma (PANC[1]) and mice fibroblasts (3T3) in concentrations of 5, 10, 25 and 50 μM. Methyl 4-(4-amidoaryl)-3-methoxy-thiophene-2-carboxylate **2** [8] (Fig. 1) exhibited potential anticancer activity with IC_{50} = 2.22 μM against MCF-7 and 0.72 μM against HepG2 cell lines.

Some 1,3-thiazole derivatives have been reported to be efficient in treatment of allergies [9], bacterial infection [10] and inflammation [11], and also potent anticancer agents [11–16]. Tiazofurin (**3**) (Fig. 1), inhibits IMP dehydrogenase, is a potential 1,3-thiazole-based anticancer drug [17, 18]. 1,3-Thiazole-based dasatinib (**4**) (Fig. 1) is a tyrosine kinase inhibitor used for treatment of leukemia and advanced prostate cancer [19].

Isatin (1*H*-indole-2,3-dione) has been emerged as an interesting moiety in the developing of several anticancer agents [20–26]. Indirubin (**5**) (Fig. 1) has potential inhibitory action against CDK-2 (IC_{50} = 1.0 μM) [27]. Sunitinib (**6**, Sutent®; Fig. 1) is an active isatin derivative that acts as an inhibitor for multi-targeted tyrosine kinase used for the management of gastrointestinal stromal tumors and metastatic renal-cell carcinoma [28, 29].

Based on the above and in continuation of our studies in the synthesis of new heterocycles as potential anticancer agents [30–35], herein we present synthesis of a new series of thiophenes **6a–6e** and 1,3-thiazole derivatives **13a–13f** and **15a–15e**, and evalua-

[1] The text was submitted by the authors in English.

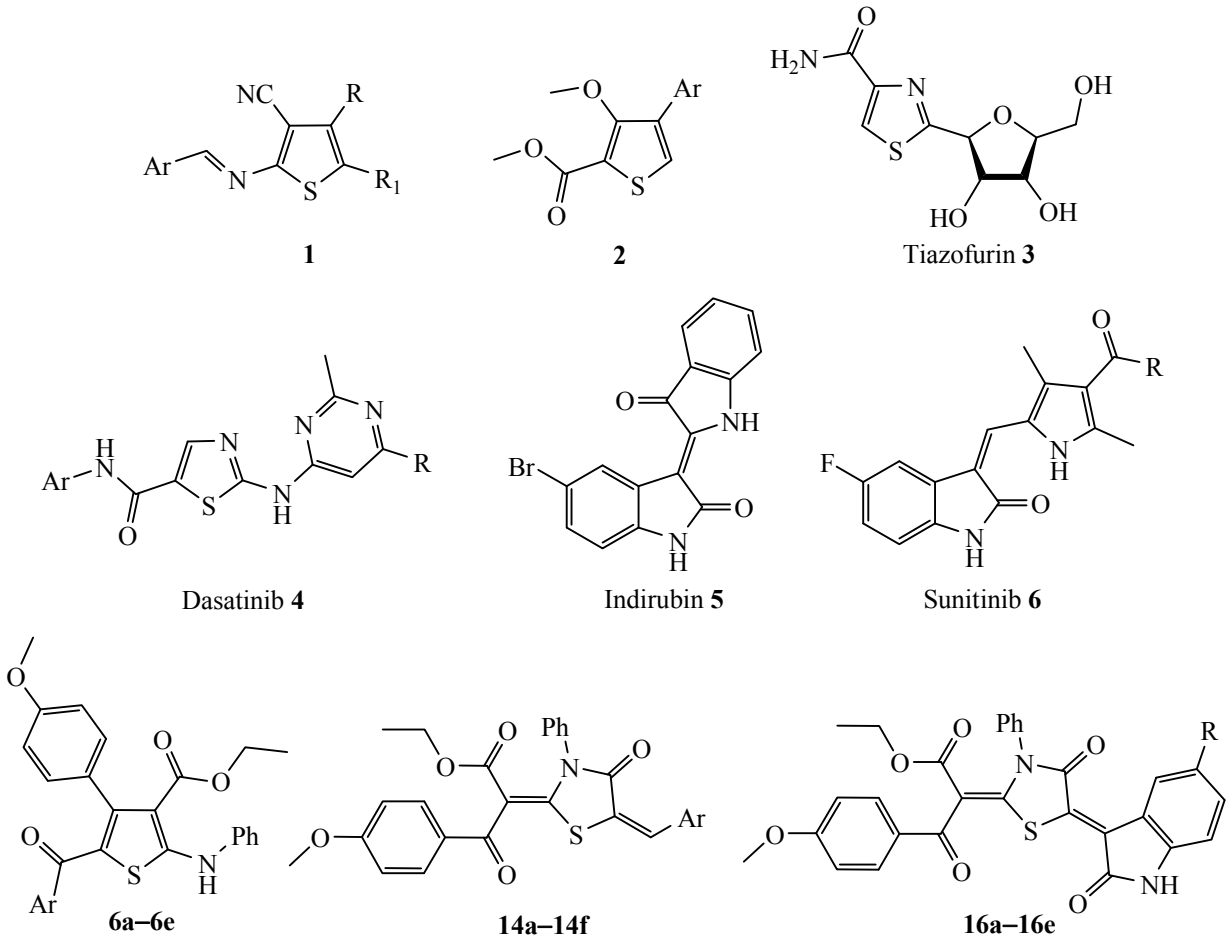


Fig. 1. The structure of compounds **1–6**, the newly synthesized thiophenes **6a–6e** and 1,3-thiazole derivatives **14a–14f** and **16a–16e.**

tion of their activity against human liver carcinoma (HepG-2) and human breast adenocarcinoma (MCF-7).

RESULTS AND DISCUSSION

The present study targeted the synthesis of novel thiophene and 1,3-thiazolidine derivatives (Schemes 1, 2) for testing their anticancer activity against HepG-2 and MCF-7 cell lines.

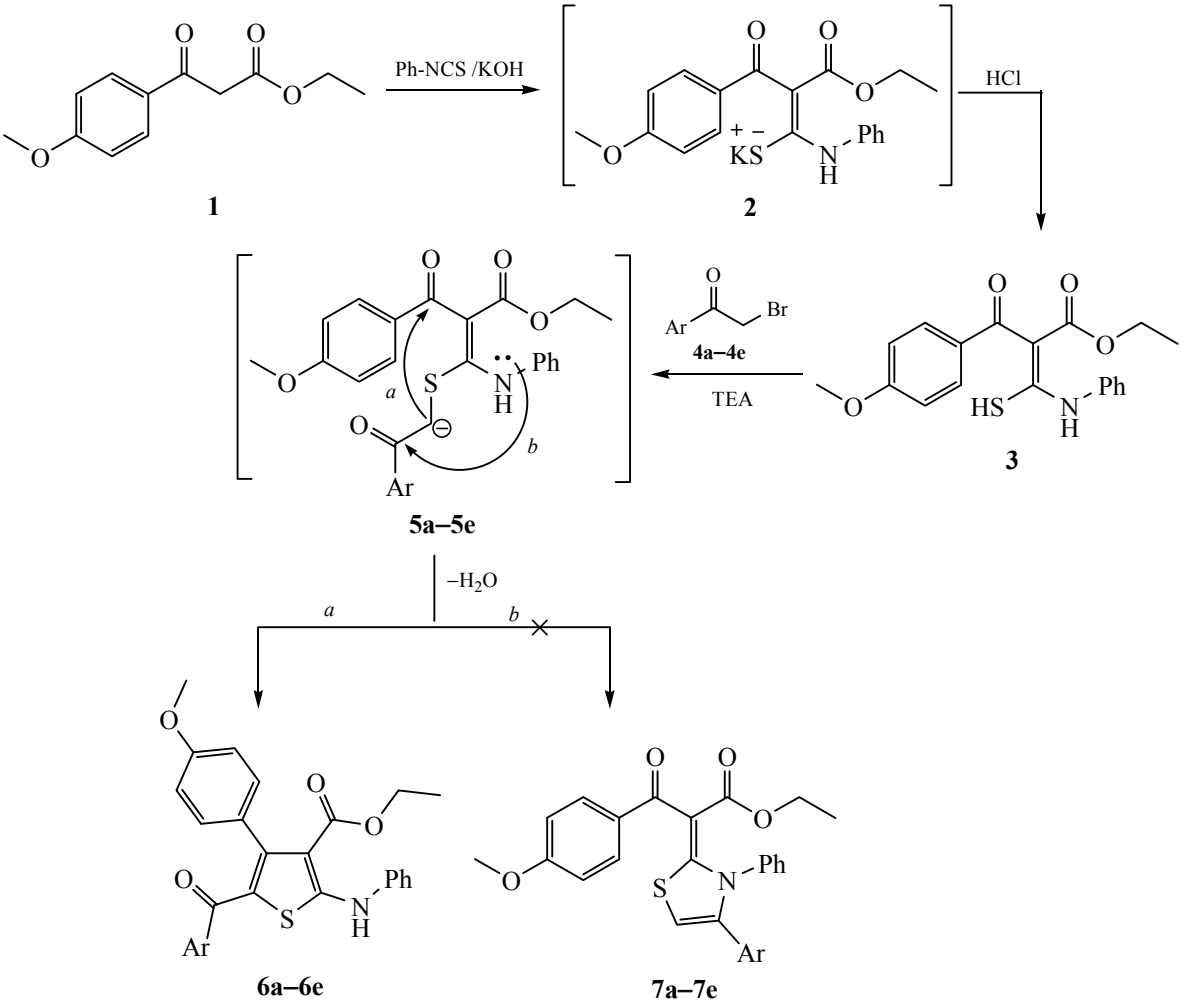
Ethyl 3-(4-methoxyphenyl)-3-oxopropanoate **1** was prepared according to the developed earkier procedures [36, 37]. The reaction of compound **1** with phenyl isothiocyanate in the presence of KOH in DMF media gave the non-isolable intermediate potassium salt **2**, which upon treatment with diluted HCl led to the corresponding ethyl 2-(4-methoxybenzoyl)-3-(phenylamino)-3-thioxopropanoate **3**, the structure of which was confirmed by ^{1}H and ^{13}C NMR spectrum.

Reaction of compound **3** with 1-aryl-2-bromoethanones **4a–4e** in ethanol, in the presence of TEA, yielded, in each case, a single product (TLC) with two possible structures (Scheme1). There were two possible routes to the reaction product via dehydration with formation of either thiophene derivatives **6a–6e** (route *a*) [38–40] or 1,3-thiazoles **7a–7e** (route *b*) [41–43].

^{1}H NMR spectra of the isolated compounds demonstrated the characteristic signal of NH in the region 10.28–10.37 ppm and disappearance of the singlet of the methylene group which supported formation of thiophenes **6a–6e**. X-Ray single crystal analysis of compound **6c** supported the absolute confirmation of thiophenes **6a–6e** (Fig. 2). Therefore, the previous reaction proceeded via "route *a*." Crystallographic data for the structure **6c** has been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers: CCDC 1538956.

Reaction of compound **3** with ethyl 2-bromoacetate (**8**) in ethanol, in the presence of TEA, afforded 1,3-

Scheme 1. Synthesis of ethyl 5-aryl-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylates (**6a–6e**).



Ar = Ph (**a**), 4-MeC_6H_4 (**b**), 4-ClC_6H_4 (**c**), 4-FC_6H_4 (**d**), 4-BrC_6H_4 (**e**).

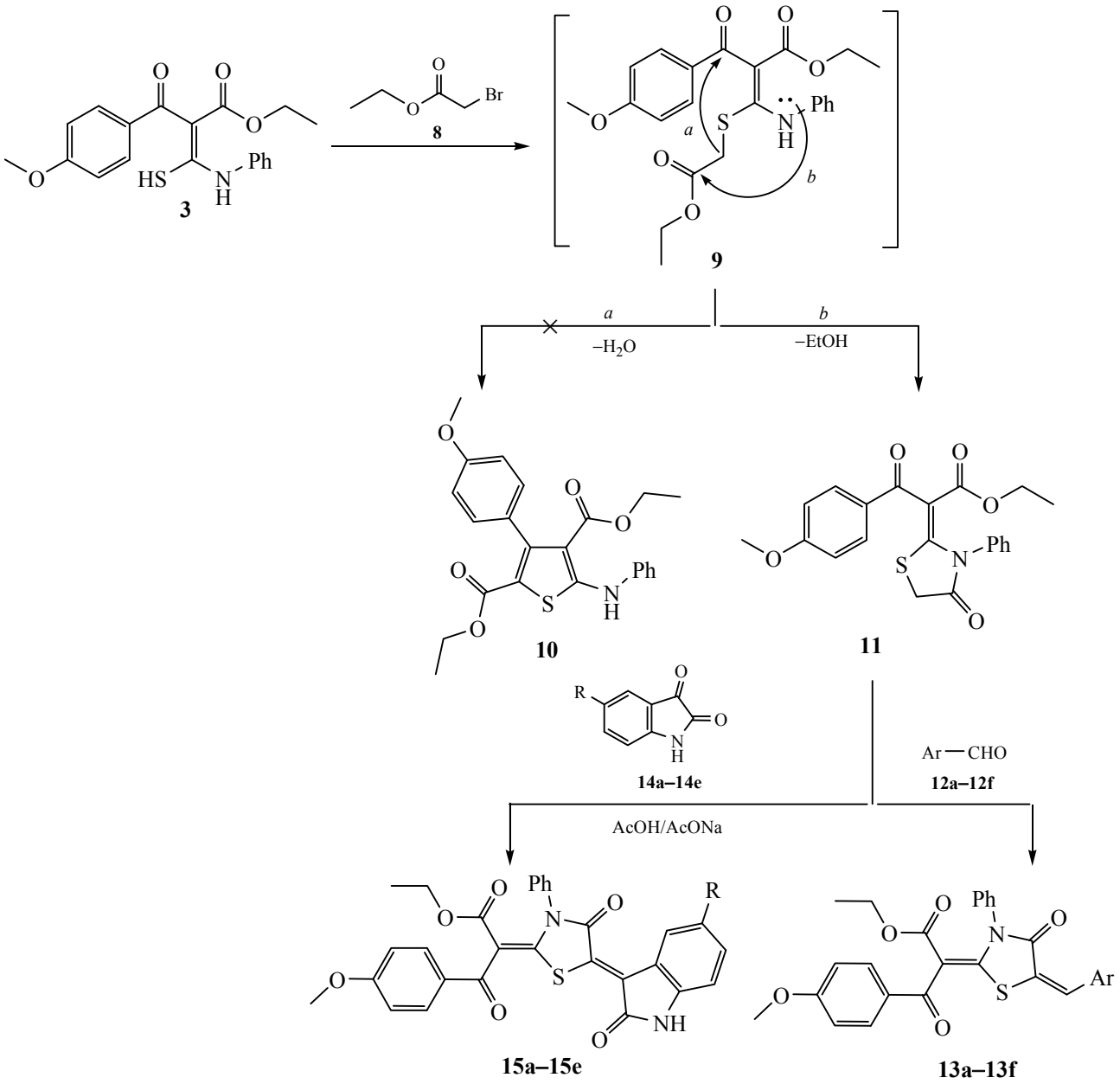
thiazolidinone **11** proceeded via "route *b*." ^{1}H NMR spectrum of compound **11** displayed the presence of a singlet of methylene protons of 1,3-thiazolidinone moiety at 4.04 ppm. ^{13}C NMR of **11** showed three signals of three C=O at 158.69 ppm (C^1 of propanoate), 165.55 ppm (C^4 of 1,3-thiazolidinone) and 188.42 (C^3 of propanoate) in addition to the signal of C^5 (CH_2) of 1,3-thiazolidinone at 31.18 ppm.

Treatment of compound **11** by aldehydes **12a–12f** in the presence of pipridine led to 5-arylidene respective derivatives **13a–13f**. ^{1}H NMR spectra of compounds **13a–13f** exhibited the presence of a singlet of Ar–CH=C< at δ 7.75 ppm. Treatment of 1,3-thizolidinone **11** with isatin dervatives **14a–4e** in glacial acetic acid and in the presences of anhydrous sodium acetate afforded the corresponding 4-oxo-5-(2-oxoindolin)-3-ylidenes **15a–15e**. ^{1}H NMR spectra of compounds **15a-f** demnstrated the presence of NH of isatin as a singlet in the range of 11.20–11.85 ppm.

Anti-tumor activity. The newly synthesized compounds **3**, **6a–6e**, **11, 13a–13f** and **15a–15e** (18 compounds), were tested *in vitro* for anti-tumor activity against HepG-2 and MCF-7 human carcinoma cell lines using the MTT assay. The IC_{50} values of all tested compounds are presented in the table. The activity of these compounds against two carcinoma cells were compared with that of Doxorubicin® (see the table). According to the accumulated data all compounds demonstrated dose-dependent anticancer activities against both cancer cells.

In general, the response of MCF-7 cancer cells was higher than that of HepG-2 cancer cells against the

Scheme 2. Synthesis of 1,3-thiazolidinones.



15: R = H (**a**), Br (**b**), Cl (**c**), F (**d**), NO_2 (**e**). **16:** Ar = Ph (**a**), 4-$MeOC_6H_4$ (**b**), 4-ClC_6H_4 (**c**), 4-FC_6H_4 (**d**), 4-$(CH_3)_2NC_6H_4$ (**e**), 2,4-diClC_6H_4 (**f**).

newly synthesized compounds. From Structure Activity Relationship (SAR) point of view, the anticancer activity of 1,3-thiazolidinones **13a–13f** and **15a–15e** was higher than that of thiophenes **6a–6e**. The unsubstituted at C^5 of isatin, ethyl 3-(4-methoxyphenyl)-3-oxo-2-[4-oxo-5-(2-oxoindolin-3-ylidene)-3-phenylthiazolidin-2-ylidene] propanoate (**15a**), was the most potent against HepG-2 cancer cells whereas *p*-MeO substituted benzylidine, ethyl 2-[5-(4-methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (**13b**) demonstrated the highest anticancer activity against MCF-7 cancer cells. The fluoro substituted at C^5 of isatin, ethyl 2-[5-(5-fluoro-2-oxoindolin-3-ylidene)-4-oxo-3-

phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (**15d**), showed anticancer activity exhibited higher activity than doxorubicin.

EXPERIMENTAL

Melting points were uncorrected and measured on an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were accumulated on a Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre, Cairo, Egypt. IR spectra were recorded (as KBr pellets) on a Perkin-Elmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt. NMR spectra were measured on a JEOL-Ex-300 MHz in DMSO-d_6 using TMS as an internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were measured on a Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV (Cairo University, Cairo, Egypt). X-Ray single crystal data were accumulated on a maXus difractometer (Bruker Nonius, Delft & MacScience, Japan).

Ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (1). The compound was synthesized according to the earlier reported method [36, 37] by the reaction of 4-methoxy acetophenone and diethyl carbonate in the presence of sodium hydride.

Ethyl 2-(4-methoxybenzoyl)-3-(phenylamino)-3-thioxopropanoate (3). A mixture of ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (**1**) (2.22 g, 10 mmol) with KOH (0.61 g, 1.1 mmol) was dissolved in DMF (25 mL) and stirred for 30 min, then phenyl isothiocyanate **2** (1.5 g, 1.1 mmol) was added. After strring for 12 h at room temperature it was poured into crushed ice and neutralized by 1 N HCl. The precipitated product was filtered off, washed with cold water, dried, and recrystallized from ethanol. Yield 91%, mp 98–100°C. IR spectrum, ν, cm^{-1}: 3181 (NH), 1737 (C=O), 1678 (C=O). ^{1}H NMR spectrum, δ, ppm: 1.17 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.85 s (3H, OCH_3), 4.15 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.03 s (1H, CH–C=S), 7.07 d (J = 9 Hz, 2H [*o*], $OMeC_6\underline{H_4}$), 7.26 t (J = 9 Hz, 1H [*p*], PhH), 7.40 t (J = 9 Hz, 2H [*m*], PhH), 7.79 d (J = 9 Hz, 2H [*o*], PhH), 7.90 d (J = 9 Hz, 2H [*o*], $OMeC_6\underline{H_4}$), 11.85 s (1H, D_2O-exchangeable, NH). ^{13}C NMR spectrum, δ, ppm: 13.81 (CH_3), 55.56 (OCH_3), 61.25 (CH_2), 68.07 (CH), 114.10 (2C), 122.87 (2C), 126.27, 128.60 (2C), 128.80, 130.52 (2C), 139.17(C^1 of Ph), 163.40 (C^4 of $OMe\underline{C_6}H_4$), 166.05 (C=O), 188.43 (C=O), 191.58 (C=S). MS, *m/z*: 358 $[M]^+$. $C_{19}H_{19}NO_4S$.

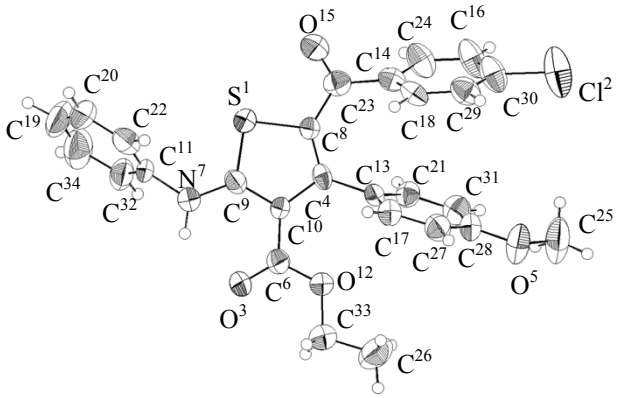


Fig. 2. X-Ray single crystal structure of ethyl 5-(4-chlorobenzoyl)-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylate (**6c**).

Synthesis of ethyl 5-aryl-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylates (6a–6e). To a mixture of ethyl 2-(4-methoxybenzoyl)-3-(phenyl-

The anticancer IC50 values of the nineteen compounds using MTT assay against two cancer types

Comp. no.	IC_{50}, μg/mL	
	HepG-2	MCF-7
3	56.56±2.9	69.46 ± 3.2
6a	84.45 ± 3.1	80.67 ± 4.6
6b	75.72 ± 4.2	73.52 ± 4.2
6c	91.30 ± 2.5	81.61 ± 3.0
6d	110.00 ± 5.1	79.03 ± 2.7
6e	84.68 ± 4.3	77.48 ± 3.1
11	86.57 ± 3.6	74.30 ± 4.2
13a	62.20 ± 2.9	70.65 ± 2.6
13b	65.93 ± 3.1	69.15 ± 1.9
13c	63.71 ± 3.2	83.09 ± 5.1
13d	68.71 ± 4.3	77.46 ± 4.2
13e	81.71 ± 5.3	83.69 ± 2.8
13f	67.13 ± 3.2	74.88 ± 3.0
15a	55.39 ± 3.2	72.01 ± 1.8
15b	72.67 ± 3.2	71.19 ± 2.4
15c	65.57 ± 2.1	72.45 ± 4.1
15d	56.574 ± 3.2	71.69 ± 2.9
15e	63.13 ± 2.7	71.34 ± 2.1
Doxorubicin	57.76 ± 2.1	74.28 ± 4.2

amino)-3-thioxopropanoate (**3**) (1 mmol) and 2-bromo-1-phenylethanone dervatives (0.357 g, 1 mmol) in absolute ethanol (25 mL) was added triethylamine (0.2 mL). The reaction mixture was refluxed for 1 h and then cooled down to room temperature. The precipitate was filtered off, dried and washed with ethanol. The crude product was recrystallized from EtOH giving compounds **6a–6e**, respectively.

Ethyl 5-benzoyl-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylate (6a). Yield 85%, mp 135–137°C. IR spectrum, ν, cm^{-1}: 3423 (NH), 1716 (C=O), 1654 (C=O). ^{1}H NMR spectrum, δ, ppm: 0.80 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.63 s (3H, OCH_3),3.99 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.55 d (J = 9 Hz, 2H [m], $OMeC_6\underline{H_4}$), 6.93–7.27 m (10H, 2H [o], $OMeC_6\underline{H_4}$ + 5H of Ph + 3H of Ar), 7.50 d (J = 9 Hz, 2H [o], ArH), 10.30 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 458 $[M]^+$. $C_{27}H_{23}NO_4S$.

Ethyl 4-(4-methoxyphenyl)-5-(4-methylbenzoyl)-2-(phenylamino)thiophene-3-carboxylate (6b). Yield 82%, mp 137–138°C. IR spectrum, ν, cm^{-1}: 3430 (NH), 1730 (C=O), 1650 (C=O). ^{1}H NMR spectrum, δ, ppm: 0.80 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 2.20 s (3H, CH_3), 3.65 s (3H, OCH_3), 3.92 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.58 d (J = 9 Hz, 2H [m], $OMeC_6\underline{H_4}$), 6.89 t (J = 9 Hz, 1H [p], PhH), 6.92 t (J = 9 Hz, 2H [m], PhH), 7.14 d (J = 9 Hz, 2H [m], ArH), 7.21–7.24 m (4H, 2H [o], PhH + 2H [o], $OMeC_6\underline{H_4}$), 7.47 d (J = 9 Hz, 2H [o], ArH), 10.28 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 472 $[M]^+$. $C_{28}H_{25}NO_4S$.

Ethyl 5-(4-chlorobenzoyl)-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylate (6c). Yield 93%, mp 140°C. IR spectrum, ν, cm^{-1}: 3435 (NH), 1735 (C=O), 1650 (C=O). ^{1}H NMR spectrum, δ, ppm: 0.79 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.66 s (3H, OCH_3), 3.97 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.55 d (J = 9 Hz, 2H [m], $OMeC_6\underline{H_4}$), 6.89 t (J = 9 Hz, 1H [p], Ph), 7.06–7.27 m (8H, 4H of PhH + 4H of $ClC_6\underline{H_4}$ + $OMeC_6\underline{H_4}$), 7.49 d (J = 9Hz, 2H [o], ClArH), 10.37 s (1H, D_2O-exchangeable, NH). ^{13}C NMR spectrum, δ, ppm: 13.29 (CH_3), 55.09 (OCH_3), 59.76 (CH_2), 109.54, 112.31 (2C), 120.61, 121.20, 121.34 (2C), 125.24, 127.26, 127.30 (2C), 129.65 (2C), 129.72 (2C), 131.18, 134.80, 137.25, 139.73 (C^1 of Ph), 147.93 (C^4 of thiophene), 158.84 (C=O of ester), 163.18 (C^4 of $OMeC_6\underline{H_4}$), 164.92 (C^2 of thiophene), 187.64 (C=O of ClAr). MS, *m/z*: 492 $[M]^+$. $C_{27}H_{22}ClNO_4S$.

Ethyl 5-(4-fluorobenzoyl)-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylate (6d). Yield 90%, mp 130°C. IR spectrum, ν, cm^{-1}: 3430 (NH), 1734 (C=O), 1655 (C=O). ^{1}H NMR spectrum, δ, ppm: 0.80 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.65 s (3H, OCH_3), 3.95 q (J = 7 Hz, 2H,$OCH_2\underline{CH_3}$), 6.58 d (J = 9 Hz, 2H [m], $OMeC_6\underline{H_4}$), 6.61–7.28 m (9H, 5H of Ph + 2H of $OMeC_6\underline{H_4}$+ 2H of FArH), 7.48 d (J = 9 Hz, 2H [o], ArH), 10.32 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 476 $[M]^+$. $C_{27}H_{22}FNO_4S$.

Ethyl 5-(4-bromobenzoyl)-4-(4-methoxyphenyl)-2-(phenylamino)thiophene-3-carboxylate (6e). Yield 86%, mp 150–152°C. IR spectrum, ν, cm^{-1}: 3430 (NH), 1735 (C=O), 1660 (C=O). ^{1}H NMR spectrum, δ, ppm: 0.79 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.67 s (3H, OCH_3), 3.92 q (J = 7 Hz, 2H, $OCH_2\underline{CH_3}$), 6.55 d (J = 9 Hz, 2H [m], $OMeC_6\underline{H_4}$), 6.89 t (J = 9 Hz, 1H [p], Ph), 7.05 t (J = 9 Hz, 2H, m-Ph), 7.08–7.27 m (6H, 2H [o], PhH + 2H [m], BrPhH + 2H [o], $OMeC_6\underline{H_4}$), 7.49 d (J = 9Hz, 2H [o], Br-ArH), 10.37 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 537 $[M]^+$. $C_{27}H_{22}BrNO_4S$.

Synthesis of ethyl 3-(4-methoxyphenyl)-3-oxo-2-(4-oxo-3-phenylthiazolidin-2-ylidene) propanoate (11). To a mixture of ethyl 2-(4-methoxybenzoyl)-3-(phenylamino)-3-thioxopropanoate (**3**) (3.57 g, 10 mmol) and ethyl 2-bromoacetate (**9**) (1.67 g, 10 mmol) in absolute ethanol (25 mL), TEA (0.2 mL) was added. The reaction mixture was refluxed for 4 h, then cooled down to room temperature. The precipitate was filtered off, dried and washed with ethanol. The crude product was recrystallized from EtOH to give the compound **11**. Yield 65%, mp 179°C. IR spectrum, ν, cm^{-1}: 1723–1689 (3C=O). ^{1}H NMR spectrum, δ, ppm: 0.88 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.81 s (3H, OCH_3), 4.0 q (J = 7 Hz, 2H, $OCH_2\underline{CH_3}$), 4.04 s (2H, CH_2 of 1,3-thiazolidinone), 6.82–7.35 m (7H, 2H [m] $OMeC_6\underline{H_4}$ + 5H Ph), 7.35(d, J = 9 Hz, 2H [o], $OMeC_6\underline{H_4}$). ^{13}C NMR spectrum, δ, ppm: 13.88 (CH_3 of ester), 31.18 (C^5 of 1,3-thiazolidinone (CH_2)), 55.42 (OCH_3), 59.90 (CH_2 of ester), 104.69 (C^2 of propanoate), 113.15 (C^3 and C^5 of $MeO\underline{C}_6H_4$), 128.02, 128.19 (2C), 128.87, 129.75, 129.91, 130.66 (2C), 134.75 (C^1 of Ph), 158.69 (C^1 of propanoate), 162.96 (C^4 of $MeOC_6\underline{H_4}$), 165.55 (C^4, C=O of 1,3-thiazolidinone), 173.35 (C^2 of 1,3-thiazolidinone), 188.42 (C^3 of propanoate). MS, *m/z*: 398 $[M]^+$. $C_{21}H_{19}NO_5S$.

Synthesis of aryylidene-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-pyrazol-3-one (13a–13f). To a mixture of compound **11** (0.397g, 1 mmol) and the appropriate aldehyde (**12a–12e**) (1 mmol) in absolute ethanol (25 mL), piperidine (0.2 mL) was added. The reaction mixture was refluxed for 1 h, then cooled down to

room temperature. The precipitate was filtered off, dried and washed with ethanol. The crude product was recrystallized from EtOH to afford the respective compounds **13a–13f**.

Ethyl 2-(-5-benzylidene-4-oxo-3-phenylthiazolidin-2-ylidene)-3-(4-methoxyphenyl)-3-oxopropanoate (13a). Yield 83%, mp 215°C. IR spectrum, ν, cm^{-1}: 1725–1671 (3C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.82 s (3H, OCH_3), 4.02 q (J = 7 Hz, 2H, $OCH_2\underline{CH_3}$), 6.84–7.65 m (12H, 2H [*m*] $OMeC_6\underline{H_4}$ + 5H PhH + 5H Ar), 7.74 s (1H, –CH=C<), 7.77 d (J = 9 Hz, 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z*: 486 $[M]^+$. $C_{28}H_{23}NO_5S$.

Ethyl 2-[-5-(4-methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (13b). Yield 85%, mp 205°C. IR spectrum, ν, cm^{-1}: 1720–1670 (3C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.82 s (3H, OCH_3), 3.86 s (3H, OCH_3), 4.04 q (J = 7 Hz, 2H,$OC\underline{H_2}CH_3$), 6.84 d (J = 9 Hz, 2H [*m*] $OMeC_6\underline{H_4}$), 6.86–7.20 m (7H, 2H [*m*] $OMeC_6\underline{H_4}$ + 5H PhH), 7.36 d (J = 9 Hz, 2H [*o*] $OMeC_6\underline{H_4}$), 7.71 s (1H, –CH=C<), 7.74 d (J = 9 Hz, 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z*: 516 $[M]^+$. ($C_{29}H_{25}NO_6S$).

Ethyl 2[-5-(4-chlorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (13c). Yield 85%, mp 202°C. IR spectrum, ν, cm^{-1}: 1725-1665 (3C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.83 s (3H, OCH_3), 4.01 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.82 d (J = 9 Hz, 2H [*m*] $OMeC_6\underline{H_4}$), 6.86–7.50 m (9H, 4H $ClC_6\underline{H_4}$ + 5H PhH), 7.74 s (1H, –CH=C<), 7.80 d (J = 9 Hz, 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z*: 520 $[M]^+$. $C_{28}H_{22}ClNO_5S$.

Ethyl 2-[-5-(4-fluorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (13d). Yield 81%, mp 190°C. IR spectrum, ν, cm^{-1}: 1718–1668 (3C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.82 s (3H, OCH_3), 4.02 q (J = 7 Hz, 2H,$O\underline{CH_2}CH_3$), 6.84 d (J = 9 Hz, 2H [*m*] $OMeC_6\underline{H_4}$), 6.86–7.16 m (7H, 2H [*m*] $FC_6\underline{H_4}$ + 5H [*p*], PhH), 7.37 d (J = 9 Hz, 2H [*o*] $FC_6\underline{H_4}$), 7.75 s (1H, –CH=C<), 7.80 m (2H, 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z* 504 $[M]^+$. $C_{28}H_{22}FNO_5S$.

Ethyl 2-[-5-(4-(dimethylamino)benzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (13e). Yield 86%, mp 200°C. IR spectrum, ν, cm^{-1}: 1720–1662 (3C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.05 s [6H, $N(CH_3)_2$], 3.82 s (3H, OCH_3), 4.05 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.83 d [J = 9 Hz, 2H [*m*] $N(Me)_2C_6\underline{H_4}$}, 6.89–7.14 m (7H, 2H [*m*] $OMeC_6\underline{H_4}$ + 5H PhH), 7.36 d {J = 9 Hz, 2H [*o*] $N(Me)_2C_6\underline{H_4}$}, 7.58–7.62 m (3H, 1H, –CH=C< + 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z*: 529 $[M]^+$. $C_{30}H_{28}N_2O_5S$.

Ethyl 2-[-5-(2,4-dichlorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (13f). Yield 86%, mp 230°C. IR spectrum, ν, cm^{-1}: 1716–1662 (3C=O). ^1H NMR spectrum, δ, ppm: 0.91 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.82 s (3H, OCH_3), 4.01 q (J = 7 Hz, 2H,$O\underline{CH_2}CH_3$), 6.84 d (J = 9 Hz, 2H [*m*] $OMeC_6\underline{H_4}$), 7.00–7.18 m (7H, 2H [*m*] 2,4-$ClC_6\underline{H_4}$ + 5H PhH), 7.36 d (J = 9 Hz, 2H [*o*] 2,4-$ClC_6\underline{H_4}$), 7.74–7.88 m (3H, 1H, –CH=C<+ 2H [*o*] $OMeC_6\underline{H_4}$). MS, *m/z*: 555 $[M]^+$. $C_{28}H_{21}Cl_2NO_5S$.

Synthesis of ethyl 3-(4-methoxyphenyl)-3-oxo-2-[4-oxo-5-(2-oxoindolin-3-ylidene)-3-phenylthiazolidin-2-ylidene]propanoate derivatives (15a–15e). To a mixture of compound **11** (0.397g, 1 mmol) and the appropriate isatin (1 mmol) in glacial acetic acid (25 mL), anhydrous sodium acetate (0.25 g, 3 mmol) was added. The reaction mixture was refluxed for 4 h and then cooled down to room temperature. The precipitate was filtered off, dried and washed with ethanol. The crude product was recrystallized from EtOH/DMF to afford the respective compounds **15a–15e**.

Ethyl 3-(4-methoxyphenyl)-3-oxo-2-[4-oxo-5-(2-oxoindolin-3-ylidene)-3-phenylthiazolidin-2-ylidene]propanoate (15a). Yield 80%, mp >300°C. IR spectrum, ν, cm^{-1}: 3211 (NH), 1697–1658 (4C=O). ^1H NMR spectrum, δ, ppm: 0.92 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.82 s (3H, OCH_3), 4.01 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.84–7.40 m (12H, 4H [*o, m*], $OMeC_6\underline{H_4}$+ 3H isatin + 5H PhH), 8.65 d (J = 2 Hz, 1H of C^4 isatin), 11.19 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 527 $[M]^+$. $C_{29}H_{22}N_2O_6S$.

Ethyl 2-[-5-(5-bromo-2-oxoindolin-3-ylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (15b). Yield 83%, mp >300°C. IR spectrum, ν, cm^{-1}: 3420 (NH), 1690–1665 (4C=O). ^1H NMR spectrum, δ, ppm: 0.93 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.83 s (3H, OCH_3), 4.03 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.85–7.37 m (4H, 2H [*m*], $OMeC_6\underline{H_4}$ + 1H of C^7 isatin + 1H [*p*], PhH), 7.48 m (5H, 4H PhH + 1H of C^6 isatin), 7.50 d (J = 9 Hz, 2H [*o*], $OMeC_6\underline{H_4}$), 8.82 d (J = 2 Hz, 1H of C^4 isatin), 11.30 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 604 $[M]^+$. $C_{29}H_{21}BrN_2O_6S$.

Ethyl 2-[-5-(5-chloro-2-oxoindolin-3-ylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (15c). Yield 80%, mp >300°C. IR spectrum, ν, cm^{-1}: 3390 (NH), 1680–1669 (4C=O). ^1H NMR spectrum, δ, ppm: 0.93 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.83 s (3H, OCH_3), 4.05 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.84–7.42 m (11H, 4H [*o, m*], $OMeC_6\underline{H_4}$ + 2H of isatin + 5H PhH), 8.66 d (J = 2 Hz, 1H of C^4 isatin), 11.27 s (1H, D_2O-exchangeable, NH); MS, *m/z*: 561 $[M]^+$. $C_{29}H_{21}ClN_2O_6S$.

Ethyl 2-[-5-(5-fluoro-2-oxoindolin-3-ylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-(4-methoxyphenyl)-3-oxopropanoate (15d). Yield 80%, mp >300°C. IR spectrum, ν, cm^{-1}: 3425 (NH), 1693–1672 (4C=O). ^1H NMR spectrum, δ, ppm: 0.93 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), cm^{-1}: 3.82 s (3H, OCH_3), 4.06 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.85–7.50 m (11H, 4H [*o, m*], $OMeC_6\underline{H_4}$+ 2H of isatin + 5H PhH), 8.71 d (J = 2 Hz, 1H of C^4 isatin), 11.30 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 545 $[M]^+$. $C_{29}H_{21}FN_2O_6S$.

Ethyl 3-(4-methoxyphenyl)-2-[5-(5-nitro-2-oxoindolin-3-ylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]-3-oxopropanoate (15e). Yield 80%, mp >300°C. IR spectrum, ν, cm^{-1}:3 288 (NH), 1696–1658 (4C=O). ^1H NMR spectrum, δ, ppm: 0.94 t (J = 7 Hz, 3H, $OCH_2\underline{CH_3}$), 3.83 s (3H, OCH_3), 4.05 q (J = 7 Hz, 2H, $O\underline{CH_2}CH_3$), 6.85 d (2H, 2H [*m*], $OMeC_6\underline{H_4}$), 7.07–7.21 m (7H, 2H [*o*], $OMeC_6\underline{H_4}$ + 5H PhH), 7.39 d (J = 8.5 Hz, 1H of C^7 isatin), 8.23 d.d (J = 2.5, J = 8.5 Hz, 1H of C^6 isatin), 9.55 d (J = 2.5 Hz, 1H of C^4 isatin), 11.86 s (1H, D_2O-exchangeable, NH). MS, *m/z*: 572 $[M]^+$. $C_{29}H_{21}N_3O_8S$.

In vitro anticancer activity. Cell culture of HepG-2 (human liver carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/mL penicillin and 100 U/mL streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay. Antitumor activity against HepG-2 and MCF-7 human cancer cell lines was estimated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [44–46]. Cells were dispensed in a 96 well sterile microplate (5×10^4 cells/well), and incubated at 37°C with series of different concentrations in DMSO of each tested compound or Doxorubicin® (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 μL of MTT (2.5 mg/mL) were added to each well and then incubated for the additional 4 h. Purple formazan dye crystals were solubilized by addition of 200 μL of DMSO. Absorbance was measured at 590 nm using a SpectraMax® Paradigm® Multi-Mode microplate reader. Relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

Statistical analysis. All experiments were conducted in triplicate and repeated on three different days. All values were represented as mean ± SD. IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

CONCLUSIONS

New thiophenes and 1,3-thiazolidinones were synthesized and evaluated for their anticancer activity against HepG-2 and MCF–7. Several compounds revealed high anticancer activity. The un-substituted at C^5 of isatin **15a** was the most potent compound against HepG-2 cancer cells, whereas *p*-MeO substituted benzylidine **13b** showed the highest anticancer activity against MCF-7 cancer cells. The fluoro substituted at C^5 of isatin **15d** was more potent than doxorubicin against both HepG-2 and MCF-7 cancer cells.

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