Full Paper

Synthesis, Characterization, Antioxidant and Antitumor Evaluation of Some New Thiazolidine and Thiazolidinone Derivatives

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2-(2-Cyano-acetylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid ethyl ester (**2**) was utilized as a key intermediate for the synthesis of thiocarbamoyl derivative **3** *via* its reaction with phenyl isothiocyanate. Treatment of **3** with chloroacetyl chloride afforded thiazolidin-5-one **4**. Compound **7** reacted with different α-halo carbonyl compounds to give thiazolidine **8a**,**b**, and thiazolidin-4-one derivatives **9**. Treatment of **4** with the appropriate aromatic aldehyde and tolyl diazonium chloride afforded the corresponding thiazolidin-5-one derivatives **5a**,**b** and **6**, respectively. The thiazolidin-4-one derivative **10** was obtained *via* the reaction of compound **2** with 2-mercaptoacetic acid. Finally, the thiazoline **11** was obtained *via* the reaction of compound **2** with phenyl isothiocyanate/sulfur. The title compounds were characterized by elemental analyses and spectral data. The quantum mechanical calculations for some compounds were accomplished and subjected for antioxidant and antitumor studies, whereas, some of them exhibited promising activities.

Keywords: Antioxidant and Antitumor Activities / Benzothiophenes / PM3-semi-empirical / Thiazolidinone

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Introduction

Isothiocyanates have been used as synthetic intermediates to prepare biologically active heterocyclic compounds [1]. The biological and physiological activities of sulfur containing heterocycles may be attributed to the presence of N-C-S-fragment characteristic of thiazoles, thiazolines, and thiazolidines [2]. On the other hand, 2-aminothiophene-3-carboxylates possess a broad spectrum of biological effectiveness such as analgesic activity [3]. The corresponding 5-carboxamido-4-hydroxy-3-(*b*-D-ribofuranosyl)thiophene-2-carbonic acid derivatives were investigated as virucides and virostatic agents [4]. Furthermore, thieno[2,3-*d*]pyrimidine derivatives [5], showed interesting biological properties including antihypertensive 6], antiallergenic [7], antitumor [8], antiviral [8], anti-HIV-1 [8], and analgesic [9] activities. In view of the above biological importance and in continuation of our previous work [10–14], we aimed to the synthesis of different heterocyclic compounds from readily obtainable thiocarbamoyl intermediates. We reported herein the synthesis, antitumor, antioxidant and quantum mechanical calculations of some thiazoline and thiazolidinone derivatives using the pm3 semi-empirical molecular orbital method.

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Results and discussion

Chemistry

The Schemes 1–4 describe the synthesis of the target molecules. The starting 2-(2-cyano-acetylamino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**2**) was obtained with high yield and purity *via* cyanoacetylation of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1**) [15], with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-

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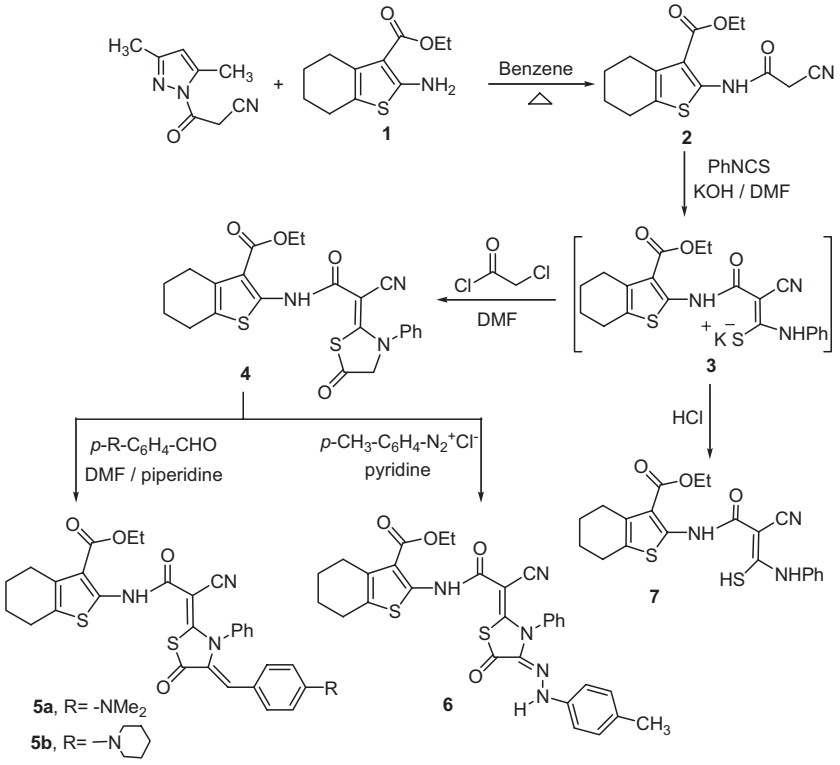

Scheme 1. Synthesis of thiazolidin-5-one and thiocarbamoyl derivatives.

oxopropanenitrile 16] through modification of the reported procedures [16, 17]. The structure of compound **2** was established by the spectral data. Whereas the IR spectrum showed a characteristic absorption band at 2258 due to cyano group and 1697, 1654 cm^{-1} corresponding to two carbonyl groups. Further, its ^{1}H-NMR displayed a singlet signal at δ 3.64 ppm, which corresponds to the methylene group of cyanoacetamide, whereas the amide NH group resonated at δ 11.9 ppm. Finally, the product was confirmed by the mass spectrum, it displayed the molecular ion peak at *m/z* 292, which matches with its molecular formula $C_{14}H_{16}N_2O_3S$.

Compound **2** reacted with phenyl isothiocyanate in dry DMF in the presence of potassium hydroxide followed by addition of chloroacetyl chloride to afford 2-(5-oxothiazolidin-2-ylidene)cyanoacetamido derivative **4** *via* the intermediate **3**. The structure of **4** was characterized by the presence of a strong absorption band at 1739 cm^{-1} in the IR spectrum, specific for the thiazolidinone; another evidence for cyclization in the ^{1}H-NMR spectrum is the presence of a singlet signal equivalent to two protons at δ 4.03 ppm, which represents the C_4 protons of the thiazolidinone ring. Formation of **4** from its precursor was confirmed by its mass spectrum which showed the molecular ion peak at *m/z* 467 in agreement with the molecular formula $C_{23}H_{21}N_3O_4S_2$.

Condensation of compound **4** with 4-(dimethylamino)-benzaldehyde or 4-(piperidin-1-yl)benzaldehyde in DMF and in the presence of a catalytic amount of piperidine gave the corresponding arylidene derivatives **5a** and **5b**. The structures of **5a** and **5b** were established on the basis on analytical and spectral data. The ^{1}H-NMR spectrum of **5a** displayed two singlet signals at δ 3.21 and 3.24 ppm due to NMe_2 group, also, **5b** showed two multiplet signals at δ 1.57–1.69, and 3.24–3.30 ppm due to piperidine ring protons.

Diazocoupling of compound **4** with *p*-tolyldiazonium chloride in pyridine afforded the corresponding 2-[4-(tolyl hydrazono)-5-oxothiazolidin-2-ylidene]cyano-acetamido derivative **6** (Scheme 1). The spectral data of compound **6** is in agreement with the proposed structure. The IR spectrum showed bands at 2202 and 1506 cm^{-1} corresponding to CN and N=N groups, respectively, its ^{1}H-NMR spectrum exhibited a singlet signal at δ 2.46 ppm due to CH_3 protons of tolyl moiety. In its mass spectrum, the compound displayed the molecular ion peak at *m/z* 585 (M^+) in agreement with the molecular formula $C_{30}H_{27}N_5O_4S_2$.

Acidification of the potassium salt **3** librated the corresponding thiocarbamoyl derivative **7**, which was established on the basis of analytical and spectral data. The IR spectrum showed six absorption bands at 3315, 3250, 2534, 2190, 1662, and 1617 cm^{-1} due to (2 NH), (SH), (CN) and two carbonyl functional groups. Its ^{1}H-NMR spectrum revealed the appearance of a three singlet signals at δ 4.61, 11.80, 12.45 ppm due to NH, NH-CO, and SH protons, respectively.

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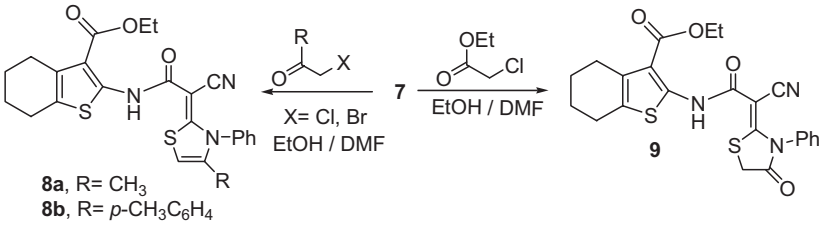


Scheme 2. Synthesis of thiazolidine and thiazolidin-4-one derivatives.

Refluxing of compound **7** in dioxane with chloroacetone, *p*-methylphenacyl chloride or ethyl chloroacetate led to the formation of thiazol-2-ylidene derivatives **8a** and **8b** and 4-oxo-3-phenyl-thiazolidin-2-ylidene derivative **9**, respectively (Scheme 2).

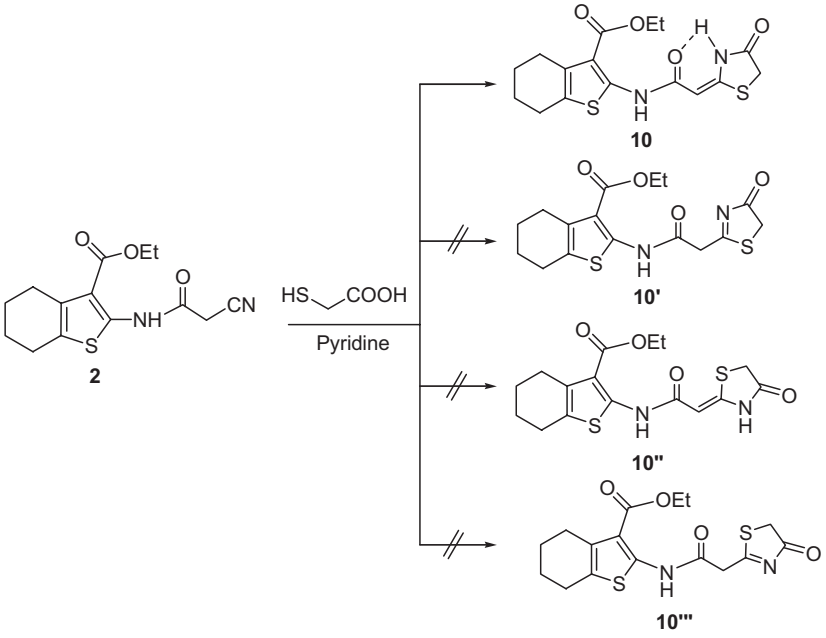
The IR spectra of compounds **8a**, **8b**, and **9** showed the presence of NH group within 3222–3158 cm^{-1} region, cyano group within 2198–2181 cm^{-1} region and two carbonyl groups within 1660–1658 and 1625–1621 cm^{-1} region. The IR of compound **9** also showed an absorption band at 1739 cm^{-1} due to the carbonyl group of thiazolidin-4-one nucleus. The ^{1}H-NMR of compounds **8a** and **8b** showed two singlet signals equivalent to three protons at δ 1.8 and 2.18 due to CH_3, aryl-CH_3, respectively, two singlet signal, equivalent to one proton at 7.02 ppm, due to C-5 proton of thiazoline nucleus and two singlet signals equivalent to one proton at δ 11.24, 11.28 ppm due to amidic proton. Moreover, the ^{1}H-NMR spectrum of compound **9** displayed a singlet signal, equivalent to two protons at δ 4.03 ppm which represents the C-5 protons of thiazolidin-4-one.

On the other hand, reaction of compound **2** with 2-mercaptoacetic acid through modification of this reported procedure [18–20], afforded the 4-oxo-thiazolidin-2-ylidene derivative **10** (Scheme 3). Compound **10** was characterized by the presence of a strong absorption band at 1700 cm^{-1} in the IR spectrum, specific for the thiazolidinone. Another evidence for cyclization, in the ^{1}H-NMR spectrum, is the presence of a singlet signal, equivalent to two protons at δ 3.74 ppm, which represents the C-5 protons of thiazolidinone nucleus.

Finally, ethyl 2-(4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**11**) was obtained by reacting **2** with sulfur and phenyl isothiocyanate following the reported reaction conditions [21], (Scheme 4). The structure of **11** was confirmed on the basis of analytical and spectral data. The IR spectrum revealed the disappearance of cyano group and the appearance of the peaks at 3421, 3397, 3299, 1681, and 1623 cm^{-1}, which correspond to NH, NH_2 and carbonyl groups, respectively.

Quantum mechanical calculation

The quantum mechanical calculations of some compounds were evaluated using the PM3 semi-empirical molecular



Scheme 3. Synthesis of 2-(4-oxothiazolidin-2-ylidene)acetamido derivatives.

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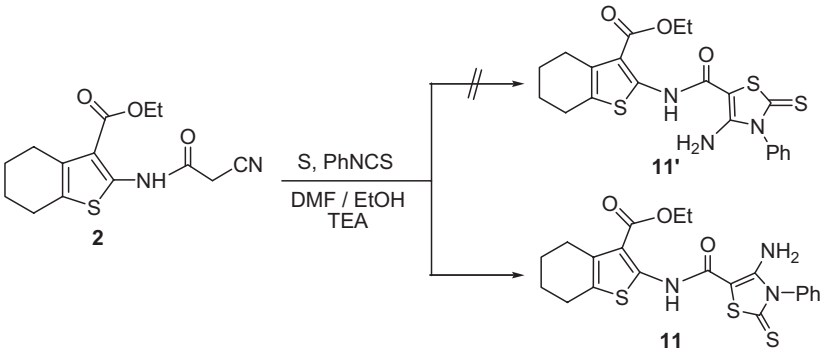

Scheme 4. Synthesis of 2-thioxo-2,3-dihydrothiazole-5-carboxamido derivatives.

orbital method, the data in Table 1 showed clearly that compounds **4**, **5a**, **7**, **8b**, and **9** were found in the *Z*-forms, whereas, *Z*-form have found to have the lower total energies than *E*-forms (Fig. 1).

Under similar behavior compounds **5b** and **8a** will be in *Z*-form. The arylidene **5a** was also found to be in the *Z,Z*-form, compound **6** was present in the *Z,E*-hydrazo form. Furthermore, compound **10** exists in the *E*-form, because this lead to the formation of hydrogen bond between NH of thiazolidinone moiety and amidic carbonyl group. Also, the ^{1}H-NMR showed a peak at δ 5.8 (s, 1H, methine proton). Finally, conformer aminothiazole **11** is more stable than conformer **11**'.

Table 1. Quantum mechanical data obtained from PM3 semi-empirical MO calculations of the configurations of different compounds

Compound No.	Configuration	Total Energy	Binding Energy
		(kcal/mol)	(kcal/mol)
4	*E*	−117 149.2348	−5756.965386
	Z	−117 150.5236	−5767.254153
5a	*Z,Z*	−148 661.7182	−7844.749003
	Z,E	−148 646.2971	−7829.327785
6	*Z,E* hydrazone	−145 858.1831	−7441.056796
	Z,Z hydrazone	−145 852.3411	−7435.214821
	Z,E azo	−145 846.8076	−7429.481371
	Z,Z azo	−145 768.7661	−7351.639824
7	*E*	−104 922.9118	−5342.308299
	Z	−104 923.2029	−5342.599391
8b	*E*	−131 628.4636	−7153.326114
	Z	−131 630.8084	−7155.670945
9	*E*	−117 155.6252	−5772.355837
	Z	−117 158.2948	−5775.025392
10		−93 274.01616	−4385.437779
10'		−93 266.47876	−4377.900382
10"		−93 270.57295	−4381.994571
10"'		−86 264.13002	−2802.127795
11		−109 223.5541	−5412.317893
11'		−109 164.7336	−5353.497415

Biological activity

ABTS Antioxidant assay

The antioxidant activity of the newly synthesized compounds was evaluated by ABTS method [22]. The data in Table 2 showed clearly that, compounds **2**, **7**, and **11** have high activity, while compound **10** exhibited moderate activity. On the other hand, compounds **5a** and **9** showed no activities and the other compounds showed weak activities. Thus, it would appear that introducing of cyanoacetamide, thiocarbamoyl, thiazolidinone, and thioxothiazole moieties enhan-

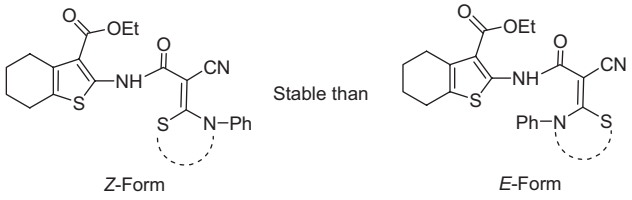

Figure 1. Geometrical isomerism of thiazole derivatives.

Table 2. ABTS Antioxidant activity assay using concentration (1 mg/mL) of benzothiophene derivatives

Compound No.	ABTS	
	Absorbance of samples	(%) Inhibition
Control of ABTS	0.491	0.000
Ascorbic acid	0.087	82.28
1	0.073	84.79
2	0.109	77.80
4	0.420	14.46
5a	0.490	0.200
5b	0.476	3.050
6	0.321	34.62
7	0.100	79.63
8a	0.313	36.25
8b	0.329	32.99
9	0.490	0.200
10	0.218	55.60
11	0.127	74.13

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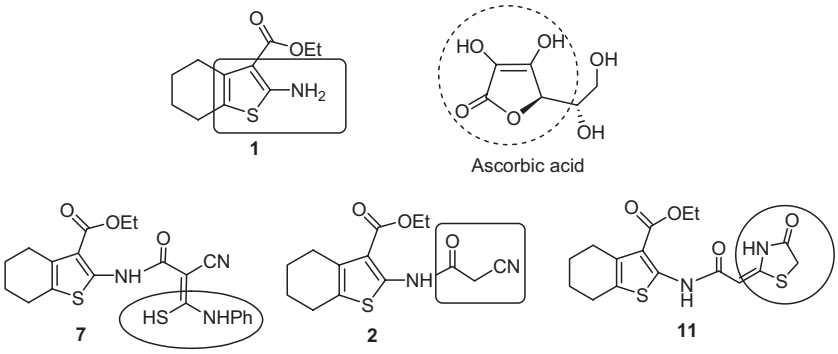

Figure 2. Structure activity relationship (SARs) of the more potent antioxidant compounds.

ces the antioxidant properties of 2-aminothiophene derivative (Fig. 2). By comparing the results obtained of antioxidant of the compounds reported in this study to their structures, the following structure activity relationship (SARs) were postulated: (i) 2-Aminothiophene derivative **1** is more potent than ascorbic acid which may be attributed to the replacement of furan moiety with the thiophene moiety. (ii) Compound **2** (*N*-substituted-2-aminothiophene) is less potent than compound **1** due to the decrease in aromaticity and conjugation. (iii) Thiocarbamoyl derivative **7** is more potent than **2** which may be attributable to presence of thiocarbamoyl moiety or may be due to oxidation of S-H to S-S and presence of further conjugation. (iv) Compound **11** is less potent than **2** which may be due to conversion of acetonitrile moiety into thioxothiazole moiety.

DNA degradative capacity

The DNA degradative capacity of the new synthesized compounds were studied using (10 mg) of each compound incubated with (100 mg) of calf thymus DNA for 1 h. The DNA was fractionated by electrophoresis in agarose matrix (Fig. 3) [23]. Compounds **2**, **4**, **6**, **8a**, and **10** showed complete degradation of the calf thymus DNA, while compounds **5a**, **b**, **8b**, and **9** showed partial degradation. The direct contact of the compounds was effective in degradation of the DNA. However, this ability on intact cells was not evaluated; it depends on the compounds diffusion through cell membranes to reach the DNA. These compounds could have a potential degradative effect on cancer cells, but further evaluations will carried out (Fig. 3).

Figure 3. The DNA degradative capacity of the new synthesized compounds.

Experimental

All melting points are recorded on Gallenkamp electric melting point apparatus and are uncorrected. The IR spectra ν (cm^{-1}) (KBr) were recorded on a Perkin Elmer Infrared Spectrophotometer Model 157. The ^{1}H-NMR spectra were obtained on a JEOL Spectrophotometer at 500 MHz, using TMS as an internal reference and DMSO-d_6 and $CDCl_3$-d_6 as solvent and were carried out in the National Research Center, Dokki, Giza, Egypt. The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment. Elemental analyses (C, H, and N) were carried out at the Microanalytical Center of Cairo Univ., Giza, Egypt. Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (**1**) was prepared according to the procedures reported in the literature [15].

*Synthesis of ethyl 2-(2-cyanoacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **2***

A mixture of **1** (2.25 g, 10 mmol) and 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (1.63 g, 10 mmol) in benzene (20 mL), was refluxed for 5 h. The solvent was evaporated under vacuum and the residue was crystallized form ethanol to give **2**.

White powder. Yield, 90%, mp: 110°C, IR (KBr): ν_{max}, cm^{-1}: 3259 (NH); 2258 (CN); 1697, 1654 (2 C=O). ^{1}H-NMR ($CDCl_3$-d_6): δ 1.36 (t, 3H, CH_3, J = 6.9), 1.77–1.78 (m, 4H, C_5-2H, C_6-2H), 2.63–2.75 (m, 4H, C_4-2H, C_7-2H), 3.64 (s, 2H, CH_2CO), 4.34 (q, 2H, CH_2O, J = 6.9), 11.92 (s, 1H, NH-CO). MS: m/z (%) = 294 (M^+ + 2, 3.2), 292 (M^+, 25.7), 206 (65.2), 178 (16.7), 151 (14.3), 123 (10.5), 91 (19.7), 68 (100). Anal. calcd. for $C_{14}H_{16}N_2O_3S$ (292.35): C, 57.52; H, 5.52; N, 9.58. Found: C, 57.63; H, 5.58; N, 9.67%.

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*Synthesis of (Z)-ethyl 2-(2-cyano-2-(5-oxo-3-phenylthiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **4***

To a cold suspension of finely divided KOH (0.11 g, 2 mmol) in dry dimethylformamide (10 mL), the cyanoacetamide derivative **2** (0.58 g, 2 mmol) followed by phenyl isothiocyanate (0.27 g, 2 mmol) was added. The mixture was stirred at room temperature for 12 h, and then cooled again to 0°C, treated with the chloroacetyl chloride (0.22 g, 2 mmol) and left to stand at room temperature for 24 h, the mixture was poured into ice cold water. The resulting precipitate was filtered off, dried and crystallized from DMF/ethanol to afford compound **4**.

White powder. Yield, 75%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3159 (NH); 2198 (CN); 1739, 1660, 1643 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.20 (t, 3H, CH_3, J = 6.9), 1.66–1.68 (m, br., 4H, C_5-2H, C_6-2H), 2.57–2.66 (m, 4H, C_4-2H, C_7-2H), 4.03 (s, 2H, C_4-2H-thiazolidinone), 4.17 (q, 2H, CH_2O, J = 6.9), 7.41–7.5 (m, 5H, Ar-H), 11.57 (s, 1H, NH-CO). MS: m/z (%) = 469 (M^+ + 2, 5.8), 468 (M^+ + 1, 9.3), 467 (M^+, 30.4), 421 (4.0), 348 (2.9), 243 (26.0), 215 (78), 179 (17.6), 132 (38.3), 77 (100). Anal. calcd. for $C_{23}H_{21}N_3O_4S_2$ (467.56): C, 59.08; H, 4.53; N, 8.99. Found: C, 59.15; H, 4.61; N, 9.07.

*General procedure for compounds **5a** and **5b***

To a well stirred solution of compound **4** (1.87 g, 4 mmol) in DMF (20 mL), TEA (0.2 mL) and 4-(dimethylamino)benzaldehyde (0.60 g, 4 mmol) or 4-(piperidin-1-yl)benzaldehyde (0.76 g, 4 mmol) were added. The reaction mixture was stirred at 80°C for 3 h. The separated crystals was filtered, dried, and recrystallized from a mixture of MeOH/DMF to give compounds **5a** and **5b**, respectively.

*Ethyl 2-((Z)-2-cyano-2-((Z)-4-(4-(dimethylamino)-benzylidene)-5-oxo-3-phenylthiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate **5a***

Purple crystals. Yield, 85%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3300 (NH); 2198 (CN); 1704, 1641, 1614 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.19 (t, 3H, CH_3, J = 6.9), 1.66–1.68 (m, br., 4H, C_5-2H, C_6-2H), 2.53–2.60 (m, 4H, C_4-2H, C_7-2H), 3.21 (s, 3H, N-Me), 3.24 (s, 3H, N-Me), 4.05 (q, 2H, CH_2O, J = 6.9), 6.84–7.98 (m, 9H, Ar-H, =CH), 11.28 (s, 1H, NH-CO). MS: m/z (%) = 600 (M^+ + 2, 3.3), 599 (M^+ + 1, 3.3), 598 (M^+, 11.1), 552 (7.2), 422 (3.3), 374 (78.4), 177 (90.2), 77 (100). Anal. calcd. for $C_{32}H_{30}N_4O_4S_2$ (598.74): C, 64.19; H, 5.05; N, 9.36. Found: C, 64.17; H, 5.01; N, 9.30.

*Ethyl 2-((Z)-2-cyano-2-((Z)-5-oxo-3-phenyl-4-(4-(piperidin-1-yl)benzylidene)thiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo[b]- thiophene-3-carboxylate **5b***

Scarlet red crystals. Yield, 88%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3300 (NH); 2202 (CN); 1702, 1660, 1648 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.20 (t, 3H, CH_3, J = 6.9), 1.57–1.69 (m, 10H, C_5-2H, C_6-2H, $C_{3,4,5}$-2H, piperidine), 2.60–2.67 (m, 4H, C_4-2H, C_7-2H), 3.24–3.30 (m, 4H, piperidine), 4.18 (q, 2H, CH_2O, J = 6.9), 7.10 (d, 2H, *p*-disubstituted benzene, J = 8.5), 7.35–7.58 (m, 5H, *N*-phenyl), 7.6 (d, 2H, *p*-disubstituted benzene, J = 8.5), 7.73 (s, 1H, =CH), 11.65 (s, 1H, NH-CO). MS: m/z (%) = 640 (M^+ + 2, 3.2), 639 (M^+ + 1, 9.7), 638 (M^+, 16.5), 592 (10.7), 414 (100), 217 (42.3), 174 (50.6), 77 (84.1). Anal. calcd. for $C_{35}H_{34}N_4O_4S_2$ (638.80): C, 65.81; H, 5.36; N, 8.77. Found: C, 65.94; H, 5.42; N, 8.84.

*Synthesis of ethyl 2-((Z)-2-cyano-2-((E)-5-oxo-3-phenyl-4-(2-p-tolylhydrazono) thiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylate **6***

To a well stirred cooled solution of *p*-toluidine (0.43 g, 4 mmol) in conc. HCl (1.5 mL) and H_2O (2 mL)], a solution of $NaNO_2$ (0.28 g, 4.1 mmol in 5 mL H_2O) was added drop wise. The above cooled diazonium solution was added slowly to a well stirred solution of **4** (1.87 g, 4 mmol) in pyridine (10 mL). The reaction mixture was stirred for 2 h. The crude product was filtered off, dried well and recrystallized from the EtOH/benzene to give **6**.

Reddish brown powder. Yield, 76%, mp: 310°C, IR (KBr): ν_{max}, cm^{-1}: 3436 (br, NH); 2202 (CN); 1704, 1643 (br, 3 C=O), 1506 (N = N). ^{1}H-NMR (DMSO-d_6): δ 1.19 (t, 3H, CH_3, J = 6.5), 1.67–1.69 (m, 4H, C_5-2H, C_6-2H), 2.46 (s, 3H, CH_3-aryl), 2.63–2.73 (m, 4H, C_4-2H, C_7-2H), 4.17 (q, 2H, CH_2O, J = 6.9), 7.04–7.47 (m, 10H, NH, hydrazo, Ar-H), 11.34 (s, 1H, NH-CO). MS: m/z (%) = 587 (M^+ + 2, 2.2), 585 (M^+, 9.5), 361 (11.7), 317 (2.6), 251 (17.1), 206 (11.7), 169 (16.5), 106 (100), 77 (78.8). Anal. calcd. for $C_{30}H_{27}N_5O_4S_2$ (585.70): C, 61.52; H, 4.65; N, 11.96. Found: C, 61.64; H, 4.73; N, 12.05.

*Synthesis of (Z)-ethyl 2-(2-cyano-3-mercapto-3-(phenylamino)acrylamido)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylate **7***

To a cold suspension of finally divided KOH (0.22 g, 4 mmol) in dry dimethylformamide (10 mL) was added the cyanoacetamide derivative **2** (1.17 g, 4 mmol) followed by phenyl isothiocyanate (0.54 g, 4 mmol). The mixture was stirred at room temperature over night, then poured into ice cold-water and acidified with 0.1 N HCl to a pH of 3 to 4. The resulting precipitate was filtered off, dried and crystallized from ethanol-benzene to give compound **7**.

Pale yellow powder. Yield, 67%, mp: 151°C, IR (KBr): ν_{max}, cm^{-1}: 3315 (2 NH); 2190 (CN); 1662, 1617 (2 C=O). ^{1}H-NMR ($CDCl_3$-d_6): δ 1.36 (t, 3H, CH_3, J = 6.9), 1.78–1.79 (m, 4H, C_5-2H, C_6-2H), 2.64–2.79 (m, 4H, C_4-2H, C_7-2H), 4.34 (q, 2H, CH_2O, J = 6.9), 4.61 (s, 1H, NH-Ph), 11.80 (s, 1H, NH-CO), 12.45 (s, 1H, SH). MS: m/z (%) = 395 (M^+ – S, 9.3), 325 (5.6), 292 (4.5), 255 (5.0), 225 (15.9), 179 (16.9), 135 (15.6), 93 (100), 77 (43.1), 66 (50). Anal. calcd. for $C_{21}H_{21}N_3O_3S_2$ (427.54): C, 58.99; H, 4.95; N, 9.83. Found: C, 59.04; H, 5.06; N, 9.90.

*General procedure for compounds **8a**, **8b**, and **9***

To a solution of compound **7** (0.86 g, 2 mmol) in dimethylformamide (20 mL), chloroacetone (0.18 g, 2 mmol), 2-bromo-1-*p*-tolyl-ethanone (0.44 g, 2 mmol) or ethyl chloroacetate (0.24 g, 2 mmol) were added. The reaction mixture was heated under reflux for 6 h, then cooled and neutralized with saturated sodium acetate solution. The resulting precipitate was filtered off, dried and crystallized from ethanol/benzene mixture for compounds **8a** and **8b** or DMF for compound **9**, respectively.

*(Z)-ethyl 2-(2-cyano-2-(4-methyl/p-tolyl-3-phenylthiazol-2(3H)-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylates **8a** and **8b***

8a: Pale yellow powder. Yield, 85%, mp: 205°C, IR (KBr): ν_{max}, cm^{-1}: 3222 (NH); 2181 (CN); 1660, 1621 (2 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.19

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(t, 3H, CH_3, $J = 6.9$), 1.66–1.67 (m, 4H, C_5-2H, C_6-2H), 1.84 (s, 3H, CH_3), 2.53–2.64 (m, 4H, C_4-2H, C_7-2H), 4.15 (q, 2H, CH_2O, $J = 6.9$), 7.02 (s, 1H, C_5-H, thiazoline), 7.32–7.55 (m, 5H, Ar-H), 11.24 (s, 1H, NH-CO). MS: m/z (%) = 467 ($M^+ + 2$, 0.9), 466 ($M^+ + 1$, 2.4), 465 (M^+, 6.7), 419 (1.2), 241 (100), 77 (14.7). Anal. calcd. for $C_{24}H_{23}N_3O_3S_2$ (465.59): C, 61.91; H, 4.98; N, 9.03. Found: C, 61.98; H, 5.07; N, 9.11.
8b: Grey powder. Yield, 82%, mp: 210°C, IR (KBr): ν_{max}, cm^{-1}: 3322 (NH); 2181 (CN); 1658, 1625 (2 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_3, $J = 6.9$), 1.67–1.68 (m, 4H, C_5-2H, C_6-2H), 2.18 (s, 3H, CH_3-aryl), 2.55–2.65 (m, 4H, C_4-2H, C_7-2H), 4.25 (q, 2H, CH_2O, $J = 6.9$), 7.02 (s, 1H, C_5-H, thiazolidine), 7.1–7.55 (m, 9H, Ar-H), 11.28 (s, 1H, NH-CO). MS: m/z (%) = 541 (M^+, 4.7), 451 (2.8), 415 (4.7), 376 (10.3), 317 (26.2), 265 (12.1), 225 (100), 206 (20.6), 179 (41.1), 151 (25.2), 135 (26.2), 119 (78.3), 91 (90.7), 77 (100). Anal. calcd. for $C_{30}H_{27}N_3O_3S_2$ (541.68): C, 66.52; H, 5.02; N, 7.76. Found: C, 66.61; H, 5.09; N, 7.84.

*(Z)-ethyl 2-(2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate **9***

Brown sheets. Yield. 76%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3158 (NH); 2198 (CN); 1739, 1656, 1645 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_3, $J = 6.9$), 1.67–1.68 (m, 4H, C_5-2H, C_6-2H), 2.57–2.66 (m, 4H, C_4-2H, C_7-2H), 4.03 (s, 2H, CH_2CO), 4.14 (q, 2H, CH_2O, $J = 6.9$), 7.41–7.5 (m, 5H, Ar-H), 11.57 (s, 1H, NH-CO). MS: m/z (%) = 469 ($M^+ + 2$, 3.5), 467 (M^+, 29.2), 421 (4.2), 347 (1.7), 234 (29.1), 215 (68.2), 179 (23.1), 132 (34.8), 77 (100). Anal. calcd. for $C_{23}H_{21}N_3O_4S_2$ (467.56): C, 59.08; H, 4.53; N, 8.99. Found: C, 59.03; H, 4.48; N, 8.92.

*Synthesis of (E)-ethyl 2-(2-(4-oxothiazolidin-2-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate **10***

2-Mercaptoacetic acid (0.18 g, 2 mmol) was added to a solution of **2** (0.58 g, 2 mmol) in pyridine (20 mL), and the reaction mixture was heated under reflux for 7 h, then left to cool to room temperature. The separated crystalline product was filtered, washed with ethanol, dried and recrystallized from ethanol/benzene mixture to give compound **10**.

Brown powder. Yield, 67%, mp: 220°C, IR (KBr): ν_{max}, cm^{-1}: 3249, 3176 (NH); 1700, 1654 (br) (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.26 (t, 3H, CH_3, $J = 6.9$), 1.67–1.68 (m, 4H, C_5-2H, C_6-2H), 2.47–2.54 (m, 4H, C_4-2H, C_7-2H), 3.74 (s, 2H, C_5-H, thiazolidinone), 4.23 (q, 2H, CH_2O, $J = 6.9$), 5.8 (s, 1H, methine proton), 10.79 (s, 1H, NH, thiazolidinone), 11.62 (s, 1H, NH-CO). MS: m/z (%) = 368 ($M^+ + 2$, 0.8), 366 (M^+, 7.7), 325 (3.0), 225 (100), 179 (78), 151 (23), 114 (16.7), 86 (25.6), 68 (58.6). Anal. calcd. for $C_{16}H_{18}N_2O_4S_2$ (366.46): C, 52.44; H, 4.95; N, 7.64. Found: C, 52.53; H, 5.04; N, 7.78.

*Synthesis of ethyl 2-(4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **11***

To a suspension of **2** (0.58 g, 2 mmol), in ethanol (20 mL) finely divided sulfur (0.06 g, 2 mmol), triethylamine (0.28 mL, 2 mmol), phenyl isothiocyanate (0.27 g, 2 mmol), and dimethylformamide (5 mL) were added. The reaction mixture was stirred at 60°C for 4 h, then left to cool at room temperature. The separated product was filtered, washed with ethanol, dried and crystallized from ethanol/benzene mixture (2:1) to give compound **11**.

Brown crystals. Yield, 90%, mp: 197°C, IR (KBr): ν_{max}, cm^{-1}: 3459, 3328 (NH); 1626, 1627 (2 C=O). ^{1}H-NMR ($CDCl_3$-d_6): δ 1.4 (t, 3H, CH_3, $J = 6.9$), 1.76–1.79 (m, br., 4H, C_5-2H, C_6-2H), 2.64–2.78 (m, 4H, C_4-2H, C_7-2H), 4.36 (q, 2H, CH_2O, $J = 6.9$), 5.96 (br, 2H, NH_2), 7.25–7.63 (m, 5H, Ar-H), 11.35 (s, 1H, NH-CO). Anal. calcd. for $C_{21}H_{21}N_3O_3S_3$ (459.60): C, 54.88; H, 4.61; N, 9.14. Found: C, 54.93; H, 4.68; N, 9.21.

Quantum mechanical calculation

The molecular total energy and preference geometry of the different configurations of the different synthesized compounds were determined by carrying out geometry optimization process of the concerned configuration using the PM3 semi-empirical Molecular orbital (MO) quantum mechanical method. The software package, Hyperchem 8.04 (Beta version) which accommodated on Pentium IV-2-8 MHz personal computer was employed.

ABTS Antioxidant assay

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation was derived from ABTS [2,2'-azino-*bis* (3-ethyl benzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 μL) with MnO_2 (3 mL, 25 mg/mL) in (5 mL) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered.

The absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was recorded at λ_{max} 734 nm. The absorbance (A_{test}) was measured upon the addition of (20 μL of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The decrease in the absorbance is expressed as %inhibition which is calculated from Eq. (1):

$$\%\text{Inhibition} = [A_{control}) - A_{test})/A_{control})] \times 100 \quad (1)$$

Ascorbic acid (20 μL, 2 mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS (Table 2).

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