Cyanoacetamide in Heterocyclic Chemistry: Synthesis, Antitumor and Antioxidant Activities of Some New Benzothiophenes

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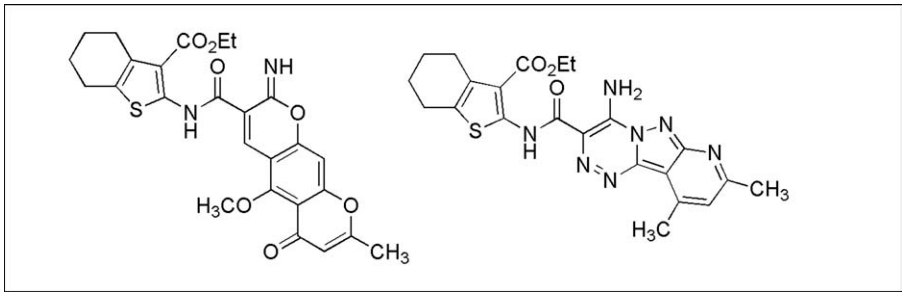
Received July 1, 2010
DOI 10.1002/jhet.634
Published online 2 August 2011 in Wiley Online Library (wileyonlinelibrary.com).



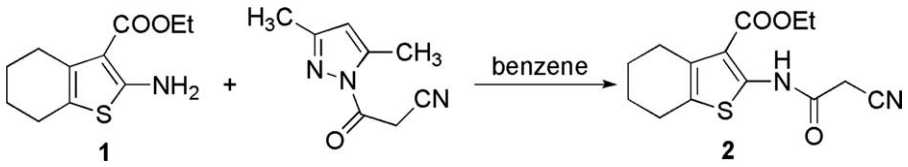
Cyanoacylation of 2-amino-tetrahydrobenzothiophene-3-carboxylate ethyl ester with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile afforded cyanoacetamide **2**. The later was utilized as key intermediate for the synthesis of 3-substituted 2-iminocoumarins **3**–**6** and acrylamides **7a**, **b** *via* Knoevenagel condensation with 2-hydroxy-1-naphthaldehyde; 2-hydroxybenzaldehyde; 1-nitrosonaphthalen-2-ol; 7-hydroxy-5-methoxy-2-methyl-4-oxo-4*H*-chromene-6-carbaldehyde; 4-dimethylamino-benzaldehyde; and 4-piperidin-1-yl-benzaldehyde in EtOH/piperidine. The derivatives **7a**, **b** did not afford the pyrazoles **8a**, **b** upon treating with phenyl hydrazine. Furthermore, coupling of **2** with 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one and 4,6-dimethyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-amine afforded the hydrazone derivatives **9** and **10**, respectively. The later derivative **10** was cyclized in acetic acid to afford the pyridopyrazolotriazine **11**. Finally, **2** was treated with dimethylformamide-dimethylacetal (DMF-DMA) to afford the dimethylaminoacrylamide **12** which underwent transamination with 4,6-dimethyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-amine to afford the pyrazole **13**. Cyclization of compound **13** in acetic acid or pyridine was unsuccessful. The antitumor and antioxidant activities of the synthesized products were evaluated; several were found to exhibit promising antioxidant activities.

J. Heterocyclic Chem., **48**, 1280 (2011).

INTRODUCTION

Substituted cyanoacetamides are important intermediates in the synthesis of a variety of agrochemicals, dyes, and pharmacologically active compounds [1]. Coumarin and their derivatives have attracted the attention of organic and medicinal chemists, as several of which are excellent scaffolds with proven multiple biological activities [2–9]. Recently the anti-inflammatory/antioxidant activities of several new coumarin derivatives with a 7-azomethine linkage have been reported [10]. The pyrazole nucleus has pronounced pharmacological applications as antianxiety, antipyretic [11], analgesic, and anti-inflammatory drugs [12–14]. Certain alkyl pyrazoles show significant bactericidal and fungicidal activities [15]. Fused heterocycles containing pyrazolopyridine systems have been prepared and found to exhibit biological and medicinal activities including anxiolytic, [16], antiviral, [17,18], antileishmanial [19], antitumor [20], and anti-inflammatory [21] profiles. In particular, thienopyridines are of special importance due to the reported biological activities [22], including antibacterial [23], anti-inflammatory [24], antiviral [25], antitumor [26], and antiparasitic [27] profiles. On the other hand, 2-amino-thiophene-3-carboxylates have been reported to possess analgesic activities [28]. The corresponding 5-carboxamido-4-hydroxy-3-(β-D-ribofuranosyl) thiophene-2-carbonic acid derivatives were investigated as virucides and virostatic agents [29]. Furthermore, thieno[2,3-*d*]pyrimidine derivatives [30], showed an interesting biological properties including antihypertensive [31], antiallergenic [32], antitumor [33], antiviral [33], anti-HIV-1 [33], and analgesic [34] activities. These biological data prompted us to synthesize some new coumarin and pyrazole derivatives incorporated with 2-amino-thiophene-3-carboxylate moiety starting from 2-amino-tetrahydrobenzothiophene-3-carboxylate ethyl ester to investigate their antitumor and antioxidant activities.

Scheme 1



RESULTS AND DISCUSSION

Chemistry. Based on the chemistry of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1**), different 3-substituted-2-iminocoumarins **3–6**, pyrazoles **9–11**, and **13** were synthesized (Schemes 1–5). The starting ethyl 2-(2-cyanoacetylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**2**) was obtained in a high yield and purity *via* cyanoacetylation of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1**) [35], with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile [36] through the modification of the reported procedures [37,38] (Scheme 1). The structure **2** was established by the spectral data. The IR spectrum showed a characteristic absorption bands at $\upsilon = 2258$ and 1654 cm^{-1} due to cyano and amidic carbonyl groups. In addition, its ^1H NMR displayed a singlet at δ 3.64 ppm, which corresponds to methylene group of cyanoacetamide, whereas the amide NH group resonates at δ 11.9 ppm. Finally, the product was confirmed by the mass spectrum that displayed the molecular ion peak at *m/z* 292, which matches with its molecular formula $C_{14}H_{16}N_2O_3S$.

Knoevenagel condensation of **2** with 2-hydroxy-1-naphthaldehyde, 2-hydroxybenzaldehyde, 1-nitrosonaphthalen-2-ol and 7-hydroxy-5-methoxy-2-methyl-4-oxo-4*H*-chromene-6-carbaldehyde in ethanol/piperidine afforded 3-substituted-2-iminocoumarines **3–6** (Scheme 2).

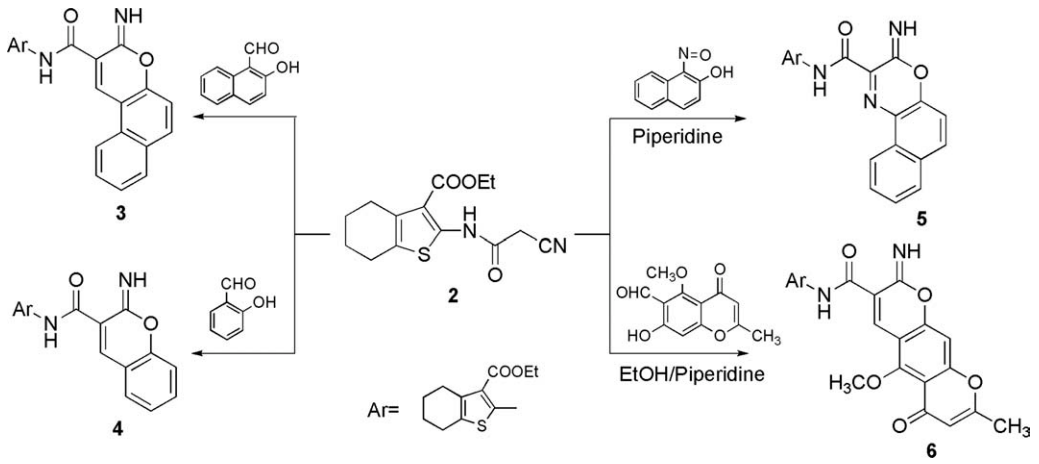
Furthermore, treatment of **2** with 4-(dimethylacrylamide)benzaldehyde and 4-(piperidin-1-yl)benzaldehyde furnished the acrylamide derivatives **7a**, **b**. Our attempts to synthesize the pyrazoles **8a**, **b** *via* treatment of **7a**, **b** with phenyl hydrazine were unsuccessful (Scheme 3).

Moreover, coupling of **2** with 1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-diazonium chloride and 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-diazonium chloride yielded the hydrazone derivatives **9** and **10**, respectively. Compound **10** was cyclized in acetic acid to give the corresponding pyrazolopyridotriazine **11** (Scheme 4).

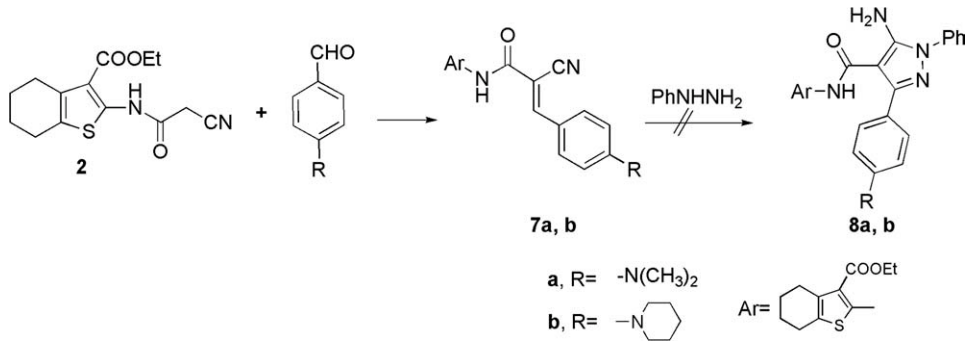
Finally, the enamine derivative **12** was synthesized *via* condensation of **2** with dimethylformamide-dimethylacetal (DMF-DMA) in dioxane. Transamination of **12** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine in dioxane/piperidine afforded ethyl 2-(2-cyano-3-(4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-ylamino)acrylamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**13**).

Our attempt to cyclize the enamine **13** to the corresponding pyrazolopyridodiazine **14** in acetic acid or pyridine was unsuccessful; compound **14** was a bioisoster of **11**. The structure assignments of new

Scheme 2



Scheme 3



compounds were based on their elemental analysis and spectral data.

The IR spectra of **3–6**, **9–11**, and **13** showed characteristic absorption bands within the $\upsilon = 1677–1624\ cm^{-1}$ region corresponding to the stretching vibration of ester and amidic groups. The absence of the absorption band corresponding to CN stretching frequency of the compound **2** clearly confirmed the formation of **3–6** and **11**.

The ^1H NMR of the newly synthesized compounds showed signals within the δ 1.23–1.35 ppm (t, 3H, CH_3), δ 4.16–4.38 ppm (q, 2H, OCH_2), δ 1.62–1.89 ppm (m, 4H, C_5—2H, C_6—2H), δ 2.68–2.91 ppm (m, 4H, C_4—2H, C_7—2H), δ 11.3–12.10 ppm (br, s, 1H, NH—CO) due to ethyl carboxylate, tetrahydrobenzene, and CO—NH moieties, respectively. Also, compounds **7a**, **b** displayed a singlet signal at δ 8.46 and 8.48 ppm, respectively, due to methine proton. Furthermore, compound **9** displayed two singlet signals at δ 2.55, 3.15 ppm corresponding to two methyl groups. Moreover, compounds **10**, **11**, and **14** showed two singlet signals within δ 2.92–3.01 and 3.09–3.19 ppm regions due to two methyl groups. Finally, compound **12** displayed three singlet signals at δ 3.16, 3.19, and 8.15 ppm attributable for dimethylaminomethine moiety.

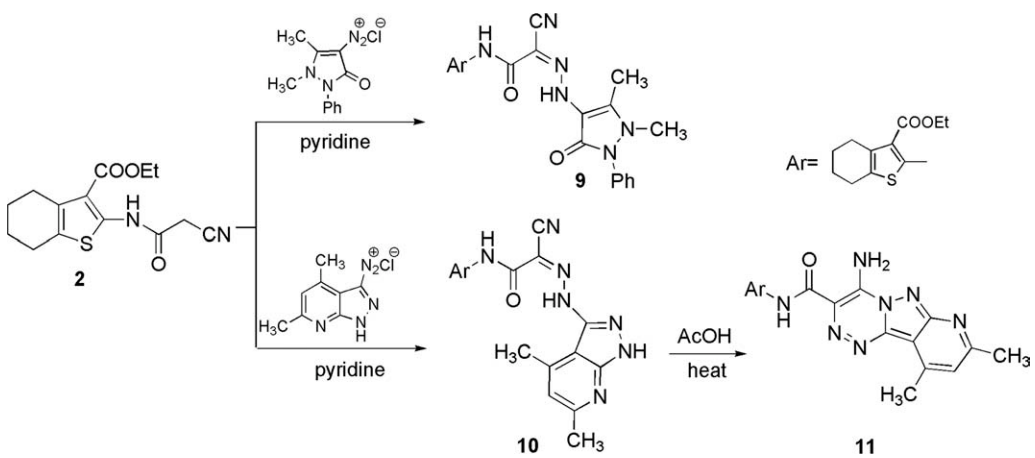
Biological activity.

Antitumor.

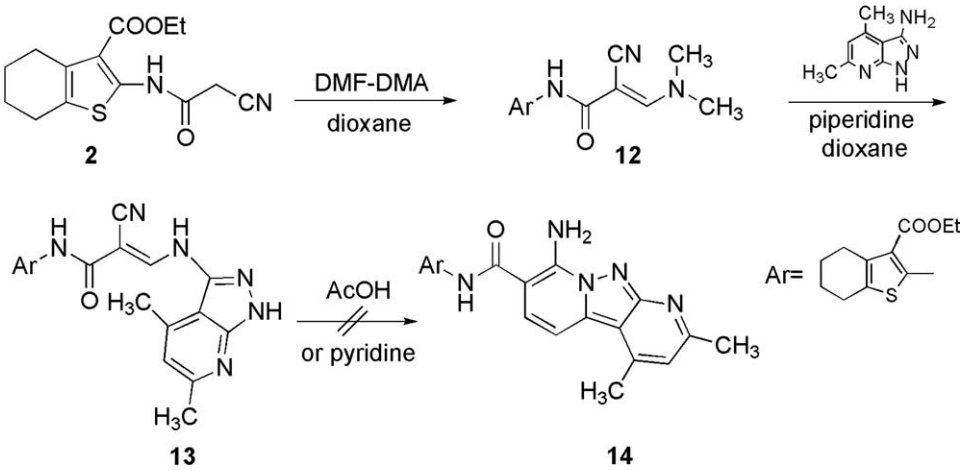
Effect of drugs on the viability of Ehrlich ascites cells in vitro. Twelve thiophene derivatives were tested for cytotoxicity against well-known established model Ehrlich ascites cells (EAC) *in vitro* [39]. The ED_{100}, ED_{50}, and ED_{25} values of the active compounds are summarized in Table 1. The data showed clearly that most of compounds have weak activities.

Antioxidant activity assay. The antioxidant activity of the newly synthesized compounds was evaluated by Lissi procedures (1999) [40]. The data clearly showed that compounds **2** and **9** have good activities, whereas compounds **7a**, **b** and **12** exhibited moderate activities. On the other hand, the other compounds showed weak activities. Thus, it has been appeared that introduction of cyanoacetamide, antipyrine, 4-dimethylaminobenzal, 4-piperidinobenzal, and dimethyl-aminomethine moieties enhances the antioxidant properties of 2-aminobenzothiophene derivative (Table 2).

Scheme 4



Scheme 5



EXPERIMENTAL

All melting points are recorded on Gallenkamp electric melting point apparatus and are uncorrected. The IR spectra (KBr) ύ (cm^{-1}) were recorded on a Perkin Elmer Infrared Spectrophotometer Model 157. The ^1H NMR spectra were recorded on a Bruker 400 MHz spectrometer using the indicated solvents using TMS as an internal reference, at the Georgia State University, Atlanta, GA. The mass spectra were recorded on 70 eV with Kratos MS equipment. Elemental analyses (C, H, and N) were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. 2-Amino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (**1**) was prepared according to the procedures reported in the literature [35].

Synthesis of 2-(2-cyano-acetylamino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylic acid ethyl ester (2). A mixture of **1** (3.15 g, 14 mmol) and 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (2.28 g, 14 mmol) in benzene (20 mL) was refluxed for 5 h. The solvent was evaporated under vacuum, and the residue was crystallized from ethanol to afford (90%) of **2**; M.p. 110°C; white powder; IR (KBr) ύ (cm^{-1}), 3259 (NH); 2258 (CN); 1697, 1654 (2C=O); ^1H NMR (400 MHz, $CHCl_3$): δ, 1.36 (t, 3H, CH_3, J = 6.9), 1.76–1.78 (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 $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58; Found C, 57.63; H, 5.58; N, 9.67.

General procedure for synthesis of 3-substituted-2-imino-coumarines 3–6 and 4-substituted benzal cyanoacetamides 7a, b. A mixture of **2** (1.46 g, 5 mmol), piperidine (0.2 mL), and 2-hydroxy-1-naphthaldehyde (0.86 g, 5 mmol); 2-hydroxybenzaldehyde (0.61 g, 5 mmol); 1-nitrosonaphthalen-2-ol (0.87 g, 5 mmol); 7-hydroxy-5-methoxy-2-methyl-4-oxo-4*H*-chromene-6-carbaldehyde (1.17 g, 5 mmol); 4-(dimethylamino)benzaldehyde (0.75 g, 5 mmol) or 4-(piperidin-1-yl)benzaldehyde (0.95 g, 5 mmol) in ethanol (15 mL) was stirred at 80°C. The

Table 1

In vitro cytotoxicity of benzothiophene derivatives (EAC, % dead).

Compound No.	% Dead		
	ED100 (μg cm^{-3})	ED50 (μg cm^{-3})	ED25 (μg cm^{-3})
5-FU	93	58	37
2	7.8	4	2.2
3	5.7	3.1	1.5
4	5.3	2.9	1.1
5	6.8	3.5	1.5
6	7	3.5	1.8
7a	4.9	2.3	1
7b	5.5	3	1.7
9	7.6	3.9	2
10	6.9	3.6	2
11	6.8	3.6	1.8
12	5.3	2.8	1.2
13	6.1	3.2	1.7

ED_{100}, ED_{50}, and ED_{25} are the effective doses at 25, 50, and 100 μL, respectively, of the compounds used. The % dead refers to the % of the dead tumor cells, and 5-FU is 5-fluorouracil as a well known cytotoxic agent.

Table 2

ABTS antioxidant activity assay of benzothiophene derivatives.

Compound No.	ABTS	
	Absorbance of samples	% Inhibition
Control of ABTS	0.506	0
Ascorbic acid	0.081	83.99
2	0.193	61.85
3	0.417	17.58
4	0.392	22.52
5	0.345	31.81
6	0.38	24.9
7a	0.3	40.71
7b	0.304	39.92
9	0.18	64.42
10	0.393	22.33
11	0.356	29.64
12	0.297	41.3
13	0.388	23.32

separated crystals was filtered, dried, and recrystallized from the appropriate solvent to give compounds **3–7a**, **b**, respectively.

Ethyl 2-(3-imino-3*H*-benzo[*f*]chromene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate (3). Reaction time 30 min, crystallized from DMF to afford (92%) of **3**; M.p. > 290°C; yellow powder; IR (KBr) ύ (cm^{-1}), 3378, 3322 (2NH), 2979, 2925 (C—H aliphatic), 1677, 1635 (2(CO), 1610 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.25 (t, 3H, CH_3, J = 6.8), 1.68–1.82 (m, 4H, C_5—2H, C_6—2H), 2.75–2.88 (m, 4H, C_4—2H, C_7—2H), 4.22 (q, 2H, CH_2O, J = 6.8), 7.43–8.03 (m, 7H, Ar-H, C_4—H, coumarin), 8.91 (s, 1H, NH), 11.62 (br, s, 1H, NH—CO); ms: (*m*/*z*, %): 413 (M^+-[Et+4H], 8.5), 366 (98.2), 151 (100), 141 (96), 140 (71.4), 139 (84), 118 (78.1), 61 (85.7). *Anal*. Calcd. for $C_{25}H_{22}N_2O_4S$: C, 67.25; H, 4.97; N, 6.27; Found C, 67.31; H, 5.03; N, 6.36.

Ethyl 2-(2-imino-2*H*-chromene-3-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (4). Reaction time 10 min, crystallized from DMF to afford (81%) of **4**; mp 230°C; yellow powder; IR (KBr) ύ (cm^{-1}), 3326 (NH), 3979, 3927 (C—H, aliphatic), 1675 (br, 2CO), 1615 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.35 (t, 3H, CH_3, J = 7.2), 1.68–1.81 (m, 4H, C_5—2H, C_6—2H), 2.79–2.86 (m, 4H, C_4—2H, C_7—2H), 4.38 (q, 2H, CH_2O, J = 7.2), 7.42–8.05 (m, 5H, Ar-H, C_4—H, coumarin), 8.98 (s, 1H, NH), 11.44 (br, s, 1H, NH—CO); ms: (*m*/*z*, %): 398 (2.4, M^++2), 396 (M^+), 363 (24.9), 317 (100), 289 (2.6), 255 (1.5), 172 (88.1), 145 (27.7), 118 (51.1), 89 (35.7), 65 (24.9). *Anal*. Calcd. for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07; Found C, 63.59; H, 5.19; N, 7.13.

Ethyl 2-(3-imino-3*H*-naphtho[2,1-*b*][1,4]oxazine-2-carboxamido)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylate (5). Reaction time 5 h, crystallized from DMF/MeOH to afford (82%) of **5**; M.p. 256°C; brown powder; IR (KBr) ύ (cm^{-1}), 3386, 3342 (2NH), 2931, 2852 (C—H aliphatic), 1660 (br, 2CO), 1619 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.30 (t, 3H, CH_3, J = 6.8), 1.69–1.89 (m, 4H, C_5—2H, C_6—2H), 2.70–2.91 (m, 4H, C_4—2H, C_7—2H), 4.27 (q, 2H, CH_2O, J = 6.8), 7.42–8.22 (m, 6H, Ar-H), 8.93 (s, 1H, NH), 11.50 (br, s, 1H, NH—CO). *Anal*. Calcd. for $C_{24}H_{21}N_3O_4S$: C, 64.41; H, 4.73; N, 9.39; Found C, 64.36; H, 4.68; N, 9.42.

Ethyl 2-(2-imino-5-methoxy-8-methyl-6-oxo-2,6-dihydropyrano[3,2-*g*]chromene-3-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (6). Reaction time 30 min, crystallized from DMF/EtOH or benzene/EtOH to afford (67%) of **6**; M.p. 274°C; reddish brown powder; IR (KBr) ύ (cm^{-1}), 3320 (NH), 2983, 2927, 2850 (C—H aliphatic), 1673 (br, 4CO), 1602 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.34 (t, 3H, CH_3, J = 6.8), 1.62–1.75 (m, 4H, C_5—2H, C_6—2H), 1.81 (s, 3H, CH_3), 2.67–2.83 (m, 4H, C_4—2H, C_7—2H), 3.92 (s, 3H, OCH_3), 4.30 (q, 2H, OCH_2, J = 6.8), 6.55 (s, 1H, C_{10}—H), 7.29 (s, 1H, C_7—H), 8.30 (s, 1H, C_4—H), 9.23 (br, s, 1H, NH), 12.20 (br, s, 1H, NH—CO); ms: (*m*/*z*, %): 475 (M^+-[Et+4H], 13.0), 429 (83.9), 284 (100), 225 (34.8), 202 (8.7), 180 (19.6), 179 (89.1), 123 (23.9), 116 (3.4), 100 (26.1), 67 (78.3), 66 (30.4). *Anal*. Calcd. for $C_{26}H_{24}N_2O_7S$: C, 61.41; H, 4.76; N, 5.51; Found C, 61.35; H, 4.82; N, 5.42.

Ethyl 2-(2-cyano-3-(4-(dimethylamino)phenyl)acrylamido)-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate (7a). Reaction time 5 h, crystallized from DMF/EtOH to afford (92%) of **7a**; M.p. 266°C; red crystals; IR (KBr) ύ (cm^{-1}), 3433 (br, NH), 2917, 2854 (C—H aliphatic), 2204, (CN), 1652 (br, 2CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.23 (t, 3H, CH_3, J = 6.8), 1.72–1.75 (m, 4H, C_5—2H, C_6—2H), 2.64–2.87 (m, 4H, C_4—2H, C_7—2H), 3.17 (s, 3H, N—CH_3), 3.19 (s, 3H, N—CH_3), 4.33 (q, 2H, OCH_2, J = 6.8), 7.15 (d, 2H, Ar-H, J = 7.6), 8.91 (d, 2H, Ar-H, J = 7.6), 8.46 (s, 1H, methine), 11.4 (br, s, 1H, NH—CO); ms: (*m*/*z*, %): 255 (M^+-[Et+4H], 100), 199 (70.6), 180 (70.6), 179 (76.5), 171 (70.6), 157 (29.4), 156 (52.9), 116 (47.1), 77 (70.6). *Anal*. Calcd. for $C_{23}H_{25}N_3O_3S$: C, 65.23; H, 5.95; N, 9.92; Found C, 65.01; H, 6.07; N, 10.02.

Ethyl 2-(2-cyano-3-(4-(piperidin-1-yl)phenyl)acrylamido)-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate (7b). Reaction time 15 min, crystallized from DMF/EtOH to afford (94%) of **7a**; mp 259°C; red crystals; IR (KBr) ύ (cm^{-1}), 3428 (br, NH), 2917, 2853 (C—H aliphatic), 2204 (CN), 1650 (br, 2CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.33 (t, 3H, CH_3, J = 7.2), 1.66–1.85 (m, 10H, C_5—2H, C_6—2H, $3CH_2$, piperidine), 2.69–2.84 (m, 4H, C_4—2H, C_7—2H), 3.47–3.60 (m, 4H, $2CH_2$, piperidine), 4.34 (q, 2H, OCH_2, J = 6.8), 7.66 (d, 2H, Ar-H, J = 7.6), 8.15 (d, 2H, Ar-H, J = 7.6), 8.48 (s, 1H, methine), 12.10 (br, s, 1H, NH—CO); ms: (*m*/*z*, %): 417 (M^+-[OEt+3H], 48.1), 355 (33.3), 239 (29.6), 225 (11.1), 174 (100), 143 (33.3), 81 (25.9), 66 (37). *Anal*. Calcd. for $C_{26}H_{29}N_3O_3S$: C, 67.36; H, 6.31; N, 9.06; Found C, 67.21; H, 6.37; N, 9.10.

General procedure for synthesis of 4,5,6,7-tetrahydrobenzo[*b*]thiophenes (9) and (10). To a well-stirred cooled solution of 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (1.02 g, 5 mmol) or 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine [41] (0.81 g, 5 mmol) in concentrated HCl (3 mL), a solution of $NaNO_2$ (0.35 g, 5.1 mmol in 5-mL H_2O) was added drop wise. The above cooled diazonium solution was added slowly to a well-stirred solution of **2** (1.46 g, 5 mmol) in pyridine (10 mL). The reaction mixture was stirred for 2 h. The crude product was filtered off, dried well, and recrystallized from EtOH-benzene to give **9** and from DMF to give **10**, respectively.

Ethyl 2-(2-cyano-2-(2-[1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl]hydra-zono)acetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (9). Crystallized from EtOH/benzene to afford (77%) of **9**; M.p. 232°C; redish brown powder; IR (KBr) ύ (cm^{-1}), 3241 (br, NH), 2933, 2858 (C—H aliphatic), 2204 (CN), 1671 (br, 3CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.29 (t, 3H, CH_3, J = 6.8), 1.68–1.79 (m, 4H, C_5—2H, C_6—2H), 2.55 (s, 3H, CH_3), 2.63–2.77 (m, 4H, C_4—2H, C_7—2H), 3.15 (s, 3H, N—CH_3), 4.16 (q, 2H, CH_2O, J = 6.8), 7.15–7.51 (m, 5H, Ar-H), 10.2 (br, s, 1H, NH), 11.30 (br, s, 1H, NH—CO). *Anal*. Calcd. for $C_{25}H_{26}N_6O_4S$: C, 59.27; H, 5.17; N, 16.59; Found C, 59.32; H, 5.25; N, 16.51.

Ethyl 2-(2-cyano-2-(2-(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-yl)hydrazono) acetamido)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate (10). Crystallized from DMF to afford (69%) of **10**; M.p. > 320°C; reddish brown powder; IR (KBr) ύ (cm^{-1}), 3386, 3257, 3203 (NH_2, NH), 3933, 2858 (C—H aliphatic), 1662, (2CO), 1629 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.28 (q, 3H, CH_3, J = 7.2), 1.68–1.75 (m, 4H, C_5—2H, C_6—2H), 2.69–2.83 (m, 4H, C_5—2H, C_6—2H), 2.95 (s, 3H, CH_3), 3.15 (s, 3H, CH_3), 4.36 (q, 3H, CH_3, J = 7.2), 7.40 (s, 1H, Ar-H), 7.07 (s, 1H, CH), 9.2 (br, s, 1H, NH), 10.2 (br, s,

1H, NH—N=C), 11.62 (br, s, 1H, NH—CO); ms: (*m/z*, %): 467 (M^++2, 8.5), 456 (M^+, 65.9), 419 (12.3), 392 (21.3), 345 (17.1), 241 (72.0), 215 (64.9), 179 (90.5), 151 (45.5), 146 (50.2), 133 (49.8), 133 (50.0), 131 (39.3), 119 (46.4), 91 (50.7), 78 (79.1), 77 (100). *Anal*. Calcd. for $C_{22}H_{23}N_7O_3S$: C, 56.76; H, 4.98; N, 21.06; Found C, 56.83; H, 5.06; N, 21.18.

Synthesis of ethyl 2-(8-amino-2,4-dimethyl-1,5,6,8a,9-pentaazafluorene-7-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (11). A suspension of **10** (2.33 g, 5 mmol) in acetic acid (10 mL) was refluxed for 7 h; the reaction mixture was poured into ice-cold water. The formed precipitate was filtered off, dried, and recrystallized from DMF to afford (80%) of **11**; M.p. > 320°C; reddish brown powder; IR (KBr) ύ (cm^{-1}), 3241 (NH), 2933, 2858 (C—H aliphatic), 2204 (CN), 1671 (br, 2CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.31 (t, 3H, CH_3, J = 6.8), 1.66–1.71 (m, 4H, C_5—2H, C_6—2H), 2.68–2.80 (m, 4H, C_5—2H, C_6—2H), 2.92 (s, 3H, CH_3), 3.19 (s, 3H, CH_3), 4.30 (q, 2H, CH_2O, J = 6.8), 7.38 (s, 1H, C—H), 10.3 (br, s, 2H, NH_2), 12.01 (br, s, 1H, NH—CO). *Anal*. Calcd. for $C_{22}H_{23}N_7O_3S$: C, 56.76; H, 4.98; N, 21.06; Found C, 56.65; H, 4.93; N, 20.11.

Synthesis of ethyl 2-(2-cyano-3-(dimethylamino)acrylamido)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylate (12). A mixture of **2** (1.46 g, 5 mmol), DMF-DMA (0.66 g, 5.5 mmol) in dioxane (20 mL) was refluxed for 3 h, cooled. and poured into ice water. The formed precipitate was filtered off and crystallized from ethanol to afford (69%) of **12**; M.p. 240°C; yellow powder; IR (KBr) ύ (cm^{-1}), 3235 (NH), 2939 (C—H aliphatic), 2184 (CN), 1668, 1624 (2CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.31 (q, 3H, CH_3, J = 7.2), 1.63–1.73 (m, C_5—2H, C_6—2H), 2.63–2.86 (m, C_5—2H, C_6—2H), 3.16 (s, 3H, N—CH_3), 3.19 (s, 3H, N—CH_3), 4.26 (q, 2H, CH_2O, J = 7.2), 8.15 (s, 1H, methine), 11.5 (br, s, 1H, NH—CO); ms: (*m/z*, %): 349 (M^++2, 1.0), 347 (M^+, 0.7), 301 (2.3), 259 (1.4), 225 (0.5), 206 (2.0), 178 (3.1), 151 (1.7), 123 (100), 80 (14.4). *Anal*. Calcd. for $C_{17}H_{21}N_3O_3S$: C, 58.77; H, 6.09; N, 12.09; Found C, 58.81; H, 6.18; N, 12.01.

Synthesis of ethyl 2-(2-cyano-3-(4,6-dimethyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-ylamino) acrylamido)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (13). A mixture of **12** (1.74 g, 5 mmol), 4,6-dimethyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-amine (0.81 g, 5 mmol), and piperidine (0.2 mL) in dioxane (20 mL) was refluxed for 5 h and cooled, and the formed precipitate was filtered and recrystallized from EtOH/DMF to afford (85%) of **13**; M.p. 258°C; yellow powder; IR (KBr) ύ (cm^{-1}), 3383, 3280, 3236 (3NH), 2937, 2885 (C—H aliphatic), 2186 (CN), 1662 (br, 2CO), 1618 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 1.33 (t, 3H, CH_3, J = 6.8), 1.69–1.73 (m, 4H, C_5—2H, C_6—2H), 2.7–2.85 (m, 4H, C_5—2H, C_6—2H), 3.01 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 4.29 (q, 2H, CH_2O, J = 6.8), 5.3 (br, s, 1H, NH), 7.07 (s, 1H, CH-Ar), 8.11 (s, 1H, methine proton), 8.71 (s, NH, pyrazole), 11.6 (br, s, 1H, NH—CO); ms: (*m/z*, %): 465 (M^++1, 1.0), 464 (M^+, 24.1), 431 (9.0), 491 (5.3), 302 (5.3), 240 (100), 183 (10.5), 179 (42.9), 151 (4.1), 116 (15.0), 78 (39.8). *Anal*. Calcd. for $C_{23}H_{24}N_6O_3S$: C, 59.47; H, 5.21; N, 18.09; Found C, 59.55; H, 5.27; N, 18.13.

Antitumor activity. Different concentrations of the tested compounds were prepared (ED_{100}, ED_{50}, and ED_{25} μg mL^{-1} DMSO). The amount of DMSO was adjusted to give a final concentration of 0.1%. Ascites fluid obtained was aseptically aspirated from the peritoneal cavity of the donor animal (National Cancer Institute, Cairo, Egypt), which contains Ehrlich cell. The cells were grown partially floating and attach in a suspension culture (RPMI 1660 medium, Sigma Chemical, St. Louis), supplemented with 10% foetal bovine serum (GIBCO, UK). They were maintained at 37°C in humidified atmosphere with 5% CO_2 for 2 h. The viability of the cell used in control experiments (DMSO only without drug) exceeded 95% as determined by microscopic examination using a hemocytometer and trypan blue stain (stain only the dead cells).

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)] and was prepared by the reaction of ABTS (60 μL) with MnO_2 (3 mL, 25 mg mL^{-1}) 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 on the addition of (20 μL of 1 mg mL^{-1}) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The inhibition ratio (%) was calculated using the following formula:

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

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

Acknowledgments. The authors are grateful to Dr. A. A. Farahat, Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, University of Mansoura, Egypt, for spectral measurements, and he is greatly acknowledged.

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