European Journal of Chemistry 5 (4) (2014) 644-651

European Journal of Chemistry
Journal homepage: www.eurjchem.com

Synthesis and antioxidant evaluation of novel sophisticated carboxamides based on 3-(ethoxycarbonyl)-4,5,6,7-tetrahydro-1-benzothiophen-2-amine

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.5.4.644-651.1125

Received: 18 July 2014
Received in revised form: 05 August 2014
Accepted: 23 August 2014
Online: 31 December 2014

KEYWORDS

Thiocarbamoyl
Cyanoacetylation
2-Aminothiophene
Antioxidant evaluation
3,5-Dimethyl-1*H*-pyrazole
3-(Ethoxycarbonyl)-4,5,6,7-tetrahydro-1-benzothiophen-2-amine

ABSTRACT

Condensation of cyanoacetamide **3** with cycloalkanones and elemental sulfur in the presence of morpholine yielded the bisthiophenes **4** and **5**. Also, its (**3**) condensation with terphthalaldehyde or coupling with *p*-phenylenedidiazonium chloride afforded compound **13** and **14**, respectively. Furthermore, cyanoacetylation of compound **4** or **5** afforded the cyanoacetamides **6** and **7**, respectively. Knoevenagel condensation of compound **7** with aromatic aldehyde afforded the arylidenes **10**, **11** and coumarin **12**, respectively. Treatment of compound **3** with CS_2 in DMF/KOH followed by alkylation reaction with ethyl bromoacetate afforded the triester derivative **16**, which gave 3-aminothiophene **17** upon heating in DMF/TEA. Moreover, refluxing of compound **23** with α-haloketones afforded 3-aminothiophenes **24** and **25**. Identity of newly synthesized compounds was established by the spectral data and novel compounds were evaluated as antioxidant agents.

1. Introduction

Substituted 2-aminothiophenes are important intermediates in the synthesis of a variety of agrochemicals, dyes and pharmacologically active compounds [1-3]. The most convergent and well-established classical approach for the preparation of 2-aminothiophenes is Gewald's method [4], which involves multicomponent condensation of a ketone with an activated nitrile and elemental sulfur in the presence of morpholine as a catalyst. 3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (**2**) is a very handy and cheap cyano acetylation reagent, which was first, synthesized and introduced in common practice in the late 1950s by Ried and Scheimer [5]. It was successfully applied for the synthesis of various *N*-alkyl and *N*-aryl cyanoacetamides [6]. Recently, Gorobets *et al*. [7], prepare a series from cyanoacetamides via refluxing of 2 with the appropriate amines in toluene. More recently, we used compound **2** for preparation of ethyl 2-(2-cyano-acetylamino)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate, **3** [8].

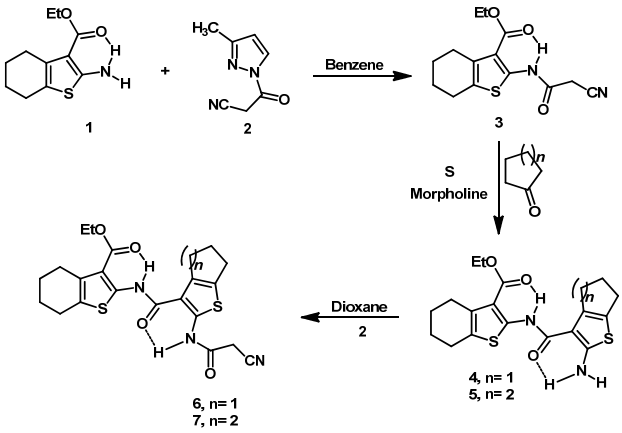
On the other hand, oxidative stress results in oxidative alteration of biological macromolecules such as lipids, proteins and nucleic acids. It is considered to play a pivotal role in the pathogenesis of aging and degenerative diseases [9-11]. In order to cope with an excess of free radicals produced upon oxidative stress, human bodies have developed sophisticated mechanisms for maintaining redox homeostasis. These protective mechanisms include scavenging or detoxification of reactive oxygen species (ROS), blocking ROS production, sequestration of transition metals, as well as enzymatic and nonenzymatic antioxidant defenses produced in the body, that is, endogenous [12,13] and others supplied with the diet, namely, exogenous ones. Among them, dietary polyphenols have been widely studied for their strong antioxidant capacities and other properties by which cell functions are regulated [14, 15]. Recently, thiophenecarbohydrazide, thienopyrazole and thienopyrimidine derivatives showed interesting biological properties including antioxidant and antitumor activities [16]. In view of the above result we use herein 3 as a building block for the construction of poly-thiophene ring systems in order to evaluate their antioxidant activity.

2. Experimental

2.1. Instrumentations

All melting points are determined on Gallenkamp electric melting point apparatus (uncorrected).

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.5.4.644-651.1125



Scheme 1

Thin layer chroma-tography (TLC) analysis was carried out on silica gel $60F_{254}$ precoated aluminum sheets. The IR spectra were recorded (KBr) on a Mattson 5000 FTIR Spectrophotometer at the Microanalytical Unit, Faculty of Science, Mansoura University $^1H/^{13}C$ NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker 400 NMR spectrometer in the indicated solvents using TMS as an internal reference, at the Georgia State University, Atlanta, Georgia, USA. The mass spectra (EI) were recorded on Kratos MS equipment at the Microanalytical Center, Cairo University, Egypt. Elemental analyses (C, H and N) were carried out at the Microanalytical Center of Cairo University, Egypt. Biological activities were carried out at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. Ethyl 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxy late (**1**) [17], ethyl 2-[(cyanoacetyl)amino]-4,5,6,7-tetrahydro-1-benzo thiophene-3-carboxylate (**3**) [8] and (*Z*)-ethyl 2-(2-cyano-3-mercapto-3-(phenylamino)acrylamido)-4, 5, 6, 7-tetrahydro benzo[*b*]thiophene-3-carboxylate (**23**) [8] were reported in the literature

2.2. Synthesis

2.2.1. Reaction of ethyl 2-[(cyanoacetyl) amino]-4,5,6,7-tetra hydro-1-benzothiophene-3-carboxylate (3) with cyclic ketones and elemental sulfur

General procedure: Morpholine (0.33 mL, 3.9 mmol), elemental sulfur (0.141 g, 4.4 mmol) and cyclopentanone (0.34 g, 4 mmol) or cyclohexanone (0.39 g, 4 mmol) were added to a solution of compound **3** (1.17 g, 4 mmol) in ethanol (20 mL). The reaction mixture was refluxed at 60-80 °C for 6 h and left to stand at room temperature. The separated crystalline product was filtered, dried and recrystallized from ethanol:benzene mixture (3:2, *v*:*v*) to give compounds **4** and **5**, respectively (Scheme 1).

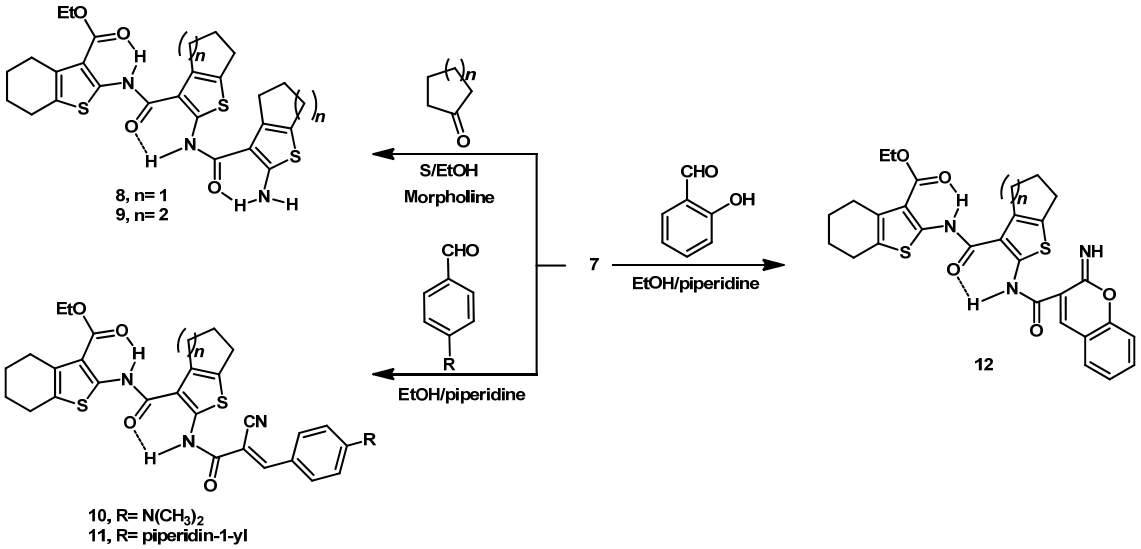
Ethyl 2-{[(2-amino-5,6-dihydro-4H-cyclopenta[b]thien-3-yl) carbonyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxy late (**4**): Color: White powder. Yield: 1.39 g, 89%. M.p.: 241 °C. FT-IR (KBr, ν, cm^{-1}): 3415, 3305, 3261 (NH_2, NH), 1673, 1616 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.30 (t, 3H, *J* = 6.8 Hz, CH_3), 1.71-1.73 (m, 4H, C5-2H, C6-2H, cyclohexane skeleton), 2.37-2.40 (m, 2H, C5-2H, cyclopentane skeleton), 2.58-2.70 (m, 6H, C4-2H, C7-2H, cyclohexane skeleton and C4-2H, cyclopentane skeleton), 3.09-3.18 (m, 2H, C6-2H, cyclopentane skeleton), 4.28 (q, 2H, *J* = 6.8 Hz, CH_2O), 7.53 (s, 2H, NH_2), 11.04 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 168.7, 165.3, 161.3, 147.0, 137.6, 129.8, 125.3, 120.3, 110.0, 100.9, 59.9, 29.28, 25.7, 23.4, 22.3, 22.2, 13.8. EI-MS (*m/z* (%)): 392 (M^++2, 3.13), 390 (M^+, 19.9), 344 (3.3), 300 (0.8), 225 (62.9), 178 (100), 166 (73.0), 150 (68.2), 138 (26.7), 122 (54.9), 109 (65.9), 104 (86.8), 90 (32.2), 76 (5.0), 65 (48.2). Anal. calcd. for $C_{19}H_{22}N_2O_3S_2$: C, 58.44; H, 5.68; N, 7.17. Found: C, 58.40; H, 5.64; N, 7.14%.

Ethyl 2-{[(2-amino-4, 5, 6, 7-tetrahydro-1-benzothien-3-yl) carbonyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxy late (**5**): Color: White powder. Yield: 1.45 g, 90%. M.p.: 200 °C. FT-IR (KBr, ν, cm^{-1}): 4421, 3397, 3299 (NH, NH_2), 1681, 1623 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.30 (t, 3H, *J* = 6.8 Hz, CH_3), 1.73-1.76 (m, 8H, $2C_5$-2H, 2C6-2H , cyclohexane skeleton), 2.50-2.59 (m, 4H, $2C_4$-2H, cyclohexane skeleton), 2.72-2.78 (m, 4H, $2C_7$-2H cyclohexane skeleton), 4.28 (q, 2H, *J* = 6.8 Hz, CH_2O), 7.34 (s, 2H, NH_2), 11.10 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 165.1, 162.4, 161.6, 147.0, 129.9, 128.3, 125.3, 116.6, 110.1, 105.1, 59.8, 25.9, 25.7, 22.3, 22.2, 13.8. EI-MS (*m/z* (%)): 406 (M^++2, 2.0), 405 (M^++1, 4.47), 404 (M+, 14.0), 325 (3.4), 225 (100), 180 (67.2), 151 (17.4), 91 (15.5), 68 (1.5). Anal. calcd. for $C_{20}H_{24}N_2O_3S_2$: C, 59.38; H, 5.98; N, 6.92. Found: C, 59.43; H, 6.05; N, 6.97%.

2.2.2. Reaction of 2-aminothiophene derivatives (4) or (5) with 3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (2)

General procedure: A mixture of compound **4** (5.40 g, 14 mmol) or **5** (5.66 g, 14 mmol) and compound **2** [18] (2.28 g, 14 mmol) in dioxane (20 mL), was refluxed for 5 h. The solvent was evaporated under vacuum and the residue was crystallized form ethanol/DMF (5:1, *v*:*v*) mixture to give compounds **6** and **7**, respectively (Scheme 1).

Ethyl 2-[({2-[(cyanoacetyl)amino]-5, 6-dihydro-4H-cyclo penta[b]thien-3-yl}carbonyl)amino]-4,5,6,7-tetrahydro-1-benzo thiophene-3-carboxylate (**6**): Color: White powder. Yield: 4.99 g, 78%. M.p.: 227 °C. FT-IR (KBr, ν, cm^{-1}): 3284, 3216 (NH), 2253 (CN), 1670, 1627 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.32 (t, 3H, *J* = 7.2 Hz, CH_3), 1.72-1.74 (m, 4H, C_5-2H, C6-2H, cyclohexane skeleton), 2.44-2.47 (m, 2H, C_5-2H, cyclopentane skeleton), 2.63-2.82 (m, 6H, C_4-2H, C_7-2H, cyclohexane skeleton and C_4-2H, cyclopentane skeleton), 3.09-3.20 (m, 2H, C_6-2H, cyclopentane skeleton), 4.23 (s, 2H, CH_2CN), 4.30 (q, 2H, *J* = 7.2 Hz, CH_2O), 11.40 (s, 1H, NH-CO), 11.64 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.2, 160.4, 149.1, 147.3, 137.8, 134.0, 130.4, 126.7, 116.0, 112.2, 111.1, 60.3, 29.0, 28.0, 27.6, 25.7, 23.5, 22.2, 22.1, 13.8. EI-MS (*m/z* (%)): 459 (M^++2, 0.6), 457 (M^+, 1.5), 295 (0.3), 251 (0.6), 233 (12.3), 225 (26.8), 205 (5.4), 178 (37.6), 165 (82.1), 151 (17.1), 104 (13.9), 91 (9.7), 67 (100). Anal. calcd. for $C_{22}H_{23}N_3O_4S_2$: C, 57.75; H, 5.07; N, 9.18. Found: C, 57.53; H, 5.01; N, 9.22%.



Scheme 2

Ethyl 2-[({2-[(cyanoacetyl)amino]-4, 5, 6, 7-tetrahydrobenzo [b]thien-3-yl}carbonyl)amino]-4, 5, 6, 7-tetrahydro-1-benzothio phene-3-carboxylate (**7**): Color: White powder. Yield: 4.95 g, 75%. M.p.: 237 °C. FT-IR (KBr, ν, cm^{-1}): 3293, 3212 (NH), 2260 (CN), 1670, 1623 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.31 (t, 3H, *J* = 6.8 Hz, CH_3), 1.74-1.80 (m, 8H, $2C_5$-2H, $2C_6$-2H, cyclohexane skeleton), 2.62-2.80 (m, 8H, $2C_4$-2H, $2C_7$-2H cyclohexane skeleton), 4.08 (s, 2H, CH_2CN), 4.31 (q, 2H, *J* = 6.8 Hz, CH_2O), 11.37 (s, 1H, NH-CO), 11.46 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 165.3, 160.8, 160.3, 145.7, 130.4, 129.2, 128.4, 126.4, 117.3, 115.0, 111.7, 60.2, 25.8, 23.6, 23.5, 22.2, 22.1, 22.0, 13.8. EI-MS (*m/z* (%)): 473 (M^++2, 0.8), 471 (M^+, 7.2), 247 (8.6), 225 (100), 206 (10.3), 179 (52.8), 151 (21.4), 91 (13.3), 68 (50.5). Anal. calcd. for $C_{23}H_{25}N_3O_4S_2$: C, 58.58; H, 5.34; N, 8.91. Found: C, 58.53; H, 5.30; N, 8.87%.

2.2.3. Reaction of ethyl-2-[({2-[(cyanoacetyl)amino] 4,5,6,7-tetrahydrobenzo [b]thien-3-yl}carbonyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (7) with cyclic ketones and elemental sulfur

General procedure: Morpholine (0.33 mL), elemental sulphur (0.141 g, 4.4 mmol) and cyclohexanone (0.4 g, 4 mmol) or cyclopentanone (0.34 g, 4 mmol) were added to solution of compound **7** (1.88 g, 4 mmol) in a mixture of ethanol:DMF (4:1, *v*:*v*, 25 mL). The reaction mixture was heated under reflux at 60-80°C for 6 h and left to cool to room temperature. The separated crystalline product was filtered, dried, and recrystallized from DMF:ethanol (2:3, *v*:*v*) to give compounds **8** and **9**, respectively (Scheme 2).

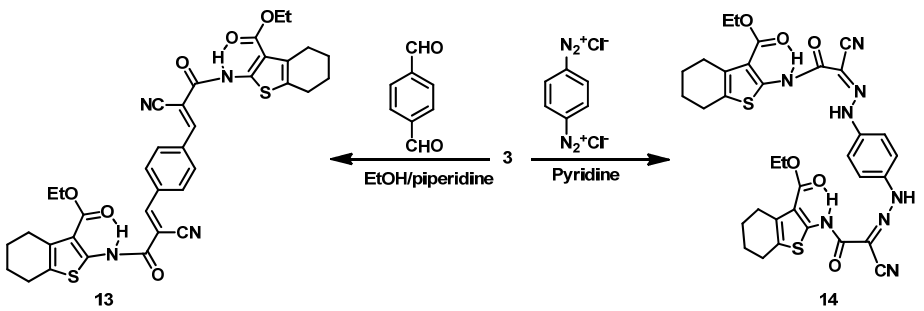
Ethyl-2-{[(2-{[(2-amino-5, 6-dihydro-4H-cyclopenta[b]thien-3-yl)carbonyl]amino}-5, 6-dihydro-4H-cyclopenta[b]-thien-3-yl) carbonyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxy late (**8**): Color: White powder. Yield: 1.50 g, 66%. M.p.: 235 °C. FT-IR (KBr, ν, cm^{-1}): 3409, 3286, 3261 (NH, NH_2), 1668, 1616 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.37 (t, 3H, *J* = 6.8 Hz, CH_3), 1.76-1.85 (m, 8H, $2C_5$-2H, $2C_6$-2H, cyclohexane skeleton), 2.56-2.87 (m, 10H, C_5-2H, cyclopentane skeleton, $2C_4$-2H, $2C_7$-2H, cyclohexane skeleton), 3.05-3.35 (m, 4H, C_4-2H, C_6-2H, cyclopentane skeleton), 4.33 (q, 2H, *J* = 6.8 Hz, CH_2O), 8.20 (s, 2H, NH_2), 11.52 (s, 1H, NH-CO), 12.44 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.5, 165.5, 160.9, 147.9, 145.1, 134.6, 134.2, 130.7, 132.0, 122.9, 120.8, 119.1, 118.0, 116.0, 115.2, 112.3, 64.5, 31.4, 31.3, 29.1, 28.2, 27.8, 25.7, 24.1, 24.0, 23.9, 14.4. EI-MS (*m/z* (%)): 570 (M^++1, 0.12), 569 (M^+, 0.13), 430 (3.4), 390 (10.7), 384 (17.7), 370 (14.9), 344 (6.42), 225 (73.5), 205 (26.8), 178 (100), 165 (76.5), 150 (64.3), 138 (25.1), 122 (38.1), 109 (29.2), 103 (27.2), 90 (25.5), 77 (34.1), 64 (27.5). Anal. calcd. for $C_{28}H_{31}N_3O_4S_3$: C, 59.02; H, 5.48; N, 7.38. Found: C, 58.98; H, 5.45; N, 7.40%.

Ethyl-2-{[(2-{[(2-amino-4, 5, 6, 7-tetrahydro-1-benzothien-3-yl)carbonyl]amino}-4,5,6, 7-tetrahydro-1-benzothien-3-yl)carbon yl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**9**): Color: White powder. Yield: 1.61 g, 69%. M.p.: 255 °C. FT-IR (KBr, ν, cm^{-1}): 3419, 3282, 3265 (NH, NH_2), 1660, 1610 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.35 (t, 3H, *J* = 6.8 Hz, CH_3), 1.71-1.82 (m, 12H, $3C_5$-2H, $3C_6$-2H, cyclohexane skeleton), 2.77-2.87 (m, 12H, $3C_4$-2H, $3C_7$-2H, cyclohexane skeleton), 4.33 (q, 2H, *J* = 6.8 Hz, CH_2O), 8.20 (s, 2H, NH_2), 11.62 (s, 1H, NH-CO), 11.80 (s, 1H, NH-CO). EI-MS (*m/z* (%)): 584 (M^++1, 0.2), 483 (0.2, M^+), 465 (0.3), 405 (0.9), 385 (0.4), 357 (0.2), 314 (0.3), 258 (1.7), 224 (8.4), 179 (100), 150 (67.0), 123 (61.2), 128 (2.1), 117 (39.1), 91 (29.6), 76 (41.2), 50 (38.1). Anal. calcd. for $C_{29}H_{33}N_3O_4S_3$: C, 59.66; H, 5.70; N, 7.20. Found: C, 59.61; H, 5.72; N, 7.18%.

2.2.4. Reaction of ethyl-2-[({2-[(cyanoacetyl)amino]-4,5,6,7-tetrahydrobenzo[b]thien-3-yl}carbonyl)amino]-4,5,6,7-tetra hydro-1-benzothiophene-3-carboxylate (7) with aromatic aldehydes

General procedure: A mixture of compound **7** (1.17 g, 2.5 mmol), 4-(dimethylamino) benzaldehyde (0.37 g, 2.5 mmol), 4-(piperidin-1-yl)benzaldehyde (0.47g, 2.5 mmol), or 2-hydroxy benzaldehyde (0.31 g, 2.5 mmol) in ethanol (15 mL) containing piperidine (0.2 mL) was stirred at 80 °C for 4 h. The separated crystals was filtered, dried and washed with hot DMF to give compounds **10**-**12**, respectively (Scheme 2).

Ethyl 2-({[2-({(2E)-2-cyano-3-[4-(dimethylamino)phenyl] prop-2-enoyl}amino)-4, 5, 6, 7-tetrahydro-1-benzothien-3-yl] carbonyl}amino)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxy late (**10**): Color: Red needle crystals. Yield: 1.43 g, 95%. M.p.: > 320 °C. FT-IR (KBr, ν, cm^{-1}): 3293, 3183 (2NH), 2196 (CN), 1688, 1614 (C=O). EI-MS (*m/z* (%)): 605 (M^++3, 29.4), 604 (M^++2, 35.3), 255 (100), 180 (70.6), 179 (76.5), 156 (52.9), 107 (29.4), 98 (35.3), 77 (70.6). Anal. calcd. for $C_{32}H_{34}N_4O_4S_2$: C, 63.76; H, 5.69; N, 9.29. Found: C, 63.69; H, 5.66; N, 9.31%.



Scheme 3

Ethyl-2-({[2-({(2E)-2-cyano-3-[4-(piperidin-1-yl)phenyl] prop-2-enoyl}amino)-4,5,6, 7-tetrahydro-1-benzothien-3-yl] carbonyl}amino)-4,5,6, 7-tetrahydro-1-benzothiophene-3-carboxylate (**11**): Color: Red crystals. Yield: 1.52 g, 95%. M.p.: 306 °C. FT-IR (KBr, ν, cm^{-1}): 3446 (br, NH), 2191(CN), 1671, 1631 (C=O), 1606 (C=N). EI-MS (*m/z* (%)): 597 (M^+-OEt, 18.5), 417 (48.1), 355 (33.3), 239 (29.6), 174 (100), 128 (18.5), 90 (14.8), 66 (37.0), 51 (18.5). Anal. calcd. for $C_{35}H_{38}N_4O_4S_2$: C, 65.39; H, 5.96; N, 8.72. Found: C, 65.41; H, 5.93; N, 8.70.

Ethyl 2-{[(2-{[(2-imino-2H-chromen-3-yl)carbonyl]amino}-4, 5, 6, 7-tetrahydro-1-benzothien-3-yl)carbonyl]amino}-4, 5, 6, 7-tetrahydro-1-benzothiophene-3-carboxylate (**12**): Color: Yellow powder. Yield: 1.28 g, 89%. M.p.: 260 °C. FT-IR (KBr, ν, cm^{-1}): 3413, 3340, 3255 (NH), 1670, 1623 (C=O). EI-MS (*m/z* (%)): 568 (M^+ -7, 0.21), 404 [M^+-3-(carbonyliminocoumarine), 0.32], 384 (M^+, 0.4), 358 (0.5), 224 (12.9), 179 (100), 164 (11.6), 151 (42.8), 134 (12.3), 123 (20.4), 117 (36.6), 97 (23.3), 86 (41.6), 74 (31.4), 50 (49.8). Anal. calcd. for $C_{30}H_{29}N_3O_5S_2$: C, 62.59; H, 5.08; N, 7.30. Found: C, 62.53; H, 5.03; N, 7.34%.

2.2.5. Synthesis of ethyl 2-((E)-2-cyano-3-(4-((E)-2-cyano-3-(3-(ethoxycarbonyl)-4,5,6, -tetrahydrobenzo-[b]thiophen-2-ylamino)-3-oxoprop-1-enyl)phenyl)acrylamido)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylate (13)

A mixture of compound **3** (0.73 g, 2.5 mmol) and terphthal aldehyde (0.17 g 1.25 mmol) in ethanol (15 mL) containing piperidine (0.2 mL) was stirred at 80 °C for 4 h. The separated crystals was filtered, dried and crystallized from DMF:ethanol (4:1, *v*:*v*) mixture to give compound **13** (Scheme 3). Color: Yellowish green powder. Yield: 0.56 g, 77%. M.p.: 239 °C. FT-IR (KBr, ν, cm^{-1}): 3184, (NH), 2195 (CN), 1729, 1677, 1621 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.34 (m, 6H, $2CH_3$), 1.75-1.82 (m, 8H, $2C_5$-2H, $2C_6$-2H, cyclohexane skeleton), 2.82-3.12 (m, 8H, $2C_4$-2H, $2C_7$-2H, cyclohexane skeleton), 4.42 (m, 4H, $2CH_2O$), 7.70-8.0 (m, 6H, Ar-H, 2CH=), 11.45 (s, 1H, NH-CO), 11.75 (s, 1H, NH-CO). EI-MS (*m/z* (%)): 679 (M^+-3, 0.1), 543 (0.2), 499 (0.2), 453 (0.2), 404 (0.3), 384 (1.2), 359 (7.8), 336 (19.3), 290 (8.9), 246 (21.5), 224 (27.4), 205 (33.4), 178 (70.0), 150 (91.3), 126 (43.9), 122 (72.9), 111 (82.8), 90(60.0), 83(72.1), 68 (100). Anal. calcd. for $C_{36}H_{34}N_4O_6S_2$: C, 63.32; H, 5.02; N, 8.21. Found: C, 63.41; H, 5.06; N, 8.23%.

2.2.6. Synthesis of ethyl-(E)-2-cyano-2-(2-(4-((E)-2-cyano-1-(3-(ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylacetamido)hydrazono)phenylhydrazono)-N-(4,5,6,7-tetra hydrobenzo [b]thiophen-2-yl)acetamido-3- carboxylate (14)

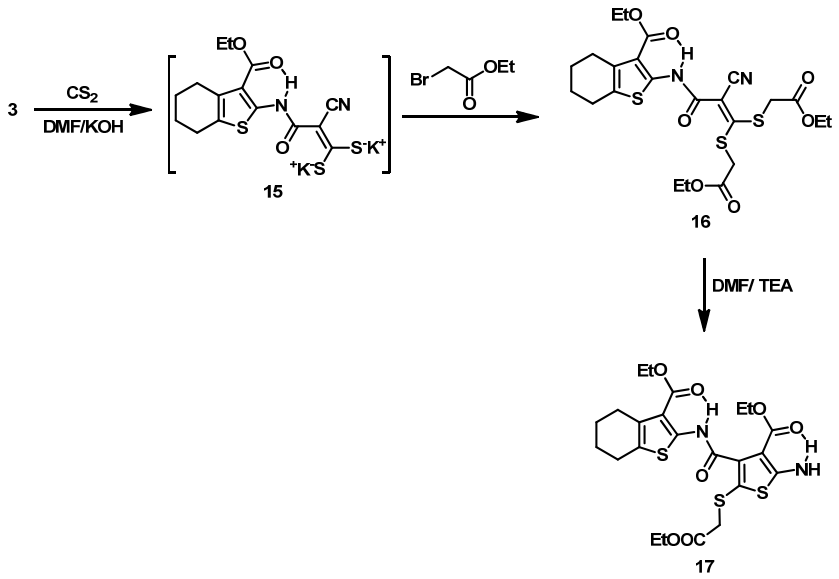
To a well stirred cooled solution of *p*-phenylenediamine (0.27 g, 2.5 mmol) in conc. HCl (3 mL), a solution of $NaNO_2$ (0.4 g, 5.8 mmol in 5 mL H_2O) was added dropwise. The above cooled diazonium salt solution was added slowly to a well stirred solution of **2** (1.46 g, 5 mmol) in pyridine (20 mL). The reaction mixture was stirred for 2 h. The crude product was filtered off, dried and recrystallize from DMF:EtOH (5:2, *v*:*v*) mixture to give compound **14** (Scheme 3). Color: Scarlet red crystals. Yield: 1.57 g, 88%. M.p.: 305 °C. FT-IR (KBr, ν, cm^{-1}): 3218, 3166 (NH), 2220, (CN), 1666, 1625 (C=O), 1488 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38-1.41 (m, 6H, $2CH_3$), 1.76-1.82 (m, 8H, $2C_5$-2H, $2C_6$-2H, cyclohexane skeleton), 2.70-2.93 (m, 8H, $2C_4$-2H, $2C_7$-2H, cyclohexane skeleton), 4.41-4.43 (m, 4H, $2CH_2O$), 7.93-8.44 (m, 4H, Ar-H), 10.45 (s, 1H, NH-CO), 10.65 (s, 1H, NH-CO), 12.45 (br, 1H, NH=N), 12.54 (br, 1H, NH=N). EI-MS (*m/z* (%)): 723 (M^++1/$2H_2O$, 0.1), 513 (M^+, 0.2), 467 (0.6), 436 (0.8), 396 (5.5), 292 (10.1), 246 (28.7), 225 (27.5), 206 (91.4), 178 (100), 150 (65.0), 121 (44.3), 115 (38.0), 104 (31.9), 90 (65.5), 67 (65.3), 64 (52.9). Anal. calcd. for $C_{34}H_{34}N_8O_6S_2$: C, 57.13; H, 4.79; N, 15.68. Found: C, 57.18; H, 4.73; N, 15.62%.

2.2.7. Synthesis of ethyl 2-(2-cyano-3,3-bis(2-ethoxy-2-oxo ethylthio)acrylamido)-4,5,6,7-tetrahydrobenzo[b]thiophene -3-carboxylate (16)

To a cold suspension of KOH (1.16 g, 20 mmole) in dry dimethylformamide (25 mL) were added the nitrile derivative **3** (1.45 g, 5 mmole) subsequently carbon disulphide (1.14 g, 15 mmol) slowly dropwise under stirring over a period of 15 min. while the temperature of the mixture was maintained at 5-10 °C. The mixture was stirred at room temperature for 12 h. Then cooled again at 0 °C, ethyl bromoacetate (1.67 g, 10 mmol) was added dropwise over a period of 10 min and left to stand at room temperature for 24 h. The mixture was poured onto ice cold-water. The resulting precipitate was filtered off, dried and crystallized from ethanol:DMF (1:5, *v*:*v*) mixture to give compound **16** (Scheme 4). Color: Yellow sheet crystals. Yield: 2.19 g, 81%. M.p.: 178 °C. FT-IR (KBr, ν, cm^{-1}): 3140 (NH), 2170 (CN), 1729, 1677, 1621 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.14 (t, 3H, *J* = 6.8 Hz, CH_3), 1.25 (t, 3H, *J* = 6.8 Hz, CH_3), 1.28 (t, 3H, *J* = 6.8 Hz, CH_3), 1.75-1.78 (m, 4H, C_5-2H, C_6-2H, cyclohexane skeleton), 2.65-2.79 (m, 4H, C_4-2H, C_7-2H, cyclohexane skeleton), 3.17 (br, 4H, $2CH_2$), 4.07 (q, 2H, *J* = 6.8 Hz, CH_2O), 4.18 (q, 2H, *J* = 6.8 Hz, CH_2O), 4.31 (q, 2H, *J* = 6.8 Hz, CH_2O), 12.66 (s, 1H, NH-CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.4, 166.5, 166.4, 163.9, 145.9, 143.1, 132.7, 127.9, 115.2, 61.4, 61.0, 60.2, 37.1, 36.7, 25.6, 23.6, 22.2, 22.1, 13.8, and 13.6. EI-MS (*m/z* (%)): 540 (M^+, 0.8), 529 (3.9), 424 (35.3), 385 (2.8), 366 (5.2), 338 (2.0), 224 (20.6), 205 (39.5), 177 (76.4), 150 (69.0), 134 (63.9), 119 (52.1), 108 (81.9), 101 (69.9), 83(76.3), 76 (100), 63 (87.3). Anal. calcd. for $C_{23}H_{29}N_2O_7S_3$: C, 51.09; H, 5.22; N, 5.18. Found: 51.15; H, 5.18; N, 5.19%.

2.2.8. Synthesis of ethyl 2-(4-amino-2-(2-ethoxy-2-oxoethyl thio)-5-(ethoxycarbonyl) thiophene-3-carboxamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (17)

A mixture of compound **16** (0.54 g, 1.0 mmol) and TEA (0.2 mL) in DMF (15 mL) was stirred at 140 °C.



Scheme 4

The mixture was cooled, poured into cooled water, the separated crystals was filtered, dried and crystallize from DMF:EtOH (1:2, *v*:*v*) mixture to give compound **17** (Scheme 4). Color: Brown powder. Yield: 0.36 g, 66%. M.p.: 192 °C. FT-IR (KBr, ν, cm^{-1}): 3411, 3280, 3261 (NH, NH_2), 1714, 1677, 1614 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.21-1.28 (m, 9H, $3CH_3$), 1.74-1.77 (m, 4H, C_5-2H, C_6-2H, cyclohexane skeleton), 2.65-2.79 (m, 4H, C_4-2H, C_7-2H, cyclohexane skeleton), 3.16 (s, 2H, CH_2), 4.26-4.31 (m, 6H, *J* = 6.8 Hz, $3CH_2O$), 5.08 (br, 2H, NH_2), 12.03 (s, 1H, NHCO). EI-MS (*m/z* (%)): 541 (M^++1, 0.2), 525 (3.5), 464 (1.1), 422 (1.9), 390 (1.7), 377 (35.2), 346 (2.6), 238 (5.3), 225 (30.2), 206 (19.3), 177 (35.2), 165 (10.1), 150 (46.5), 120 (89.1), 108 (95.3), 93 (38.7), 90 (45.1), 77 (100), 68 (41.7), 57 (86.6). Anal. calcd. for $C_{23}H_{28}N_2O_7S_3$: C, 51.09; H, 5.22; N, 5.18. Found: 51.05; H, 5.26; N, 5.15%.

2.2.9. Synthesis of ethyl 2-(2-cyano-2-(4-methyl-3-phenyl thiazol-2(3H)-ylidene)acetamido)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylate (19) and ethyl 2-(2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamido)-4,5,6,7-tetra hydrobenzo[b]thiophene-3-carboxylate (20)

To a cold suspension of finely divided KOH (0.11 g, 2 mmol) in dry dimethylformamide (10 mL), the cyanoacetamide derivative **3** (0.58 g, 2 mmol) followed by phenyl isothio cyanate (0.27 g, 2 mmol) was added. The mixture was stirred at room temperature for 12 h, and then cooled again to 5-0 °C, treated with the chloroacetone (0.18 g, 2 mmol) or ethyl bromoacetate (0.24 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 (5:2, *v*:*v*) mixture to afford compounds **19** and **20**, respectively (Scheme 5).

Ethyl 2-{[(2Z)-2-cyano-2-(4-methyl-3-phenyl-1, 3-thiazol-2(3H)-ylidene)acetyl]amino}-4, 5, 6, 7-tetrahydro-1-benzothio phene-3-carboxylate (**19**): Color: Pale yellow powder. Yield 0.59 g, 63%. M.p.: 206 °C. (Lit. [8], 205 °C).

Ethyl 2-(2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acet amido)-4, 5, 6, 7-tetrahydrobenzo[b]thiophene-3-carboxylate (**20**): Color: Brown powder. Yield: 0.51 g, 55%. M.p.: > 320 °C. (Lit. [8] > 320 °C).

2.2.10. Reaction of ethyl 2-{[(2E)-3-anilino-2-cyano-3-mercaptoprop-2-enoyl]amino}-4,5,6,7-tetrahydro-1-benzo thiophene-3-carboxylate (23) with α-halogen carbonyl compounds

Chloroacetone (0.18 g, 2 mmol) or ethyl bromoacetate was added in solution containing ethanol (10 mL), triethylamine (0.2 mL) and compound **23** (0.86 g, 2 mmole). The reaction mixture was refluxed for 3 h. The obtained product after addition of water was filtered, dried and crystallized from ethanol:benzene (1: 3, *v*:*v*) to give compounds **24** and **25**, respectively (Scheme 5).

Ethyl 2-{[(5-acetyl-4-amino-2-anilino-3-thienyl) carbonyl] amino}-4, 5, 6, 7-tetrahydro-1-benzothiophene-3-carboxylate (**24**): Color: Brown crystals. Yield 0.67 g, 69%. M.p.: 230 °C. FT-IR: 3234 (NH, NH_2, br), 1671, 1610 (C=O). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.21 (t, 3H, *J* = 7.2 Hz, CH_3), 1.74-1.90 (m, 4H, C_5-2H, C_6-2H), 2.10 (s, 3H, CH_3), 2.63-2.77 (m, 4H, C_4-2H, C_7-2H), 4.42 (q, 2H, *J* = 7.2 Hz, CH_2O), 7.1-7.5 (m, 8H, NH_2, NH, Ar-H), 11.82 (s, 1H, NH-CO). EI-MS (*m/z* (%)): 484 (M^++1, 8.9), 451 (6.9), 437 (7.6), 259 (15.0), 242 (22.0), 225 (41.0), 216 (47.9), 178 (100), 150 (66.1), 143 (37.7), 121 (41.5), 116 (37.0), 90 (36.7), 76 (64.9), 50 (25.8). Anal. calcd. For $C_{24}H_{25}N_3O_4S_2$: C, 59.61; H, 5.21; N, 8.69. Found: C, 59.56; H, 5.15; N, 8.75%.

Ethyl 2-(3-amino-2-(ethoxycarbonyl)-5-(phenylamino)thio phene-4-carboxamido)-4, 5, 6, 7-tetrahydrobenzo[b]thiophene-3-carboxylate (**25**): Color: Brown crystals. Yield 0.81 g, 79%. M.p.: 283 °C. FT-: 3366, 3200 (NH, NH_2), 1664, 1621 (C=O). EI-MS (*m/z* (%)): 515 (M^++2, 1.9), 514 (M^++1, 21.8), 468 (18.7), 422 (3.5), 394 (2.8), 288 (4.4), 243 (71.7), 225 (81.1), 215 (77.8), 178 (100), 170 (36.4), 150 (66.9), 142 (60.8), 122 (49.4), 115 (33.8), 90 (31.9), 77 (67.4). Anal. calcd. for $C_{25}H_{27}N_3O_5S_2$:C, 58.46; H, 5.30; N, 8.18. Found: C, 58.40; H, 5.36; N, 8.13%.

2.3. Antioxidant screening

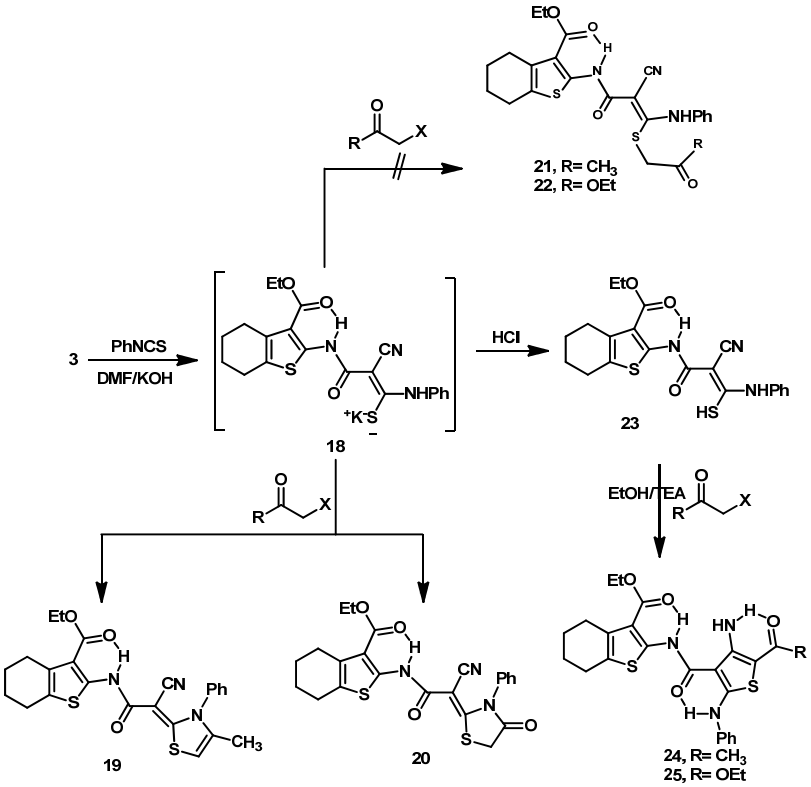
2.3.1. Superoxide anion radical scavenging assay

The assay was performed according to Nishmiki *et al*. [19], with minor modifications. Test solution (0.1 mL, 1 mg/mL of sample solution (DMSO)) in 0.1 M phosphate buffer pH = 7.4, 62.5 µL of 468 µM NADH solution, 62.5 µL of 156 µM nitroblue tetrazolium (NBT) solution and 62.5 µL of 60 µM phenazine

Table 1. Assay for superoxide anion radical scavenging and Bleomycin-dependent DNA damage (DNA)

Compound no	Antioxidant activity (Superoxide anion radical scavenging, %)	Absorbance of samples
Ascorbic Acid	66.1	0.091
1	58.6	0.078
3	48.6	0.127
4	31.2	0.080
5	35.7	0.079
6	45.7	0.113
7	61.4	0.136
8	55.8	0.125
9	41.4	0.118
10	38.6	0.157
11	10.3	0.151
12	55.7	0.102
13	6.1	0.123
14	35.6	0.145
16	58.9	0.169
17	37.1	0.134
23	57.1	0.098
24	20.0	0.100
25	18.6	0.126

* All compounds were dissolved in DMSO:MeOH (1:1, *v:v*) and tested at the final concentration of 0.1 mL of 1 mg/mL.



Scheme 5

methophosphate (PMS) solution were added to a microwell plate and incubated at room temperature for 5 min. and the abundance at 560 nm was measured against blank samples. The capacity of scavenging super oxide radicals was calculated using the following formula:

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

The (NBT+NADH+PMS) solution without sample solution was used as control.

2.3.2. Bleomycin-dependent DNA damage

The assay was performed according to Aeschlach *et al.* [20], with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and samples to be tested (0.1 mL of 1 mg/mL). L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL) (1%, *w:v*) and HCl (0.5 mL) (25%, *v:v*) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm (Table 1).

3. Results and discussion

3.1. Chemistry

Schemes 1-4 describe the syntheses of the target molecules. The starting ethyl 2-[(cyanoacetyl)amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate **3** [8] was prepared according

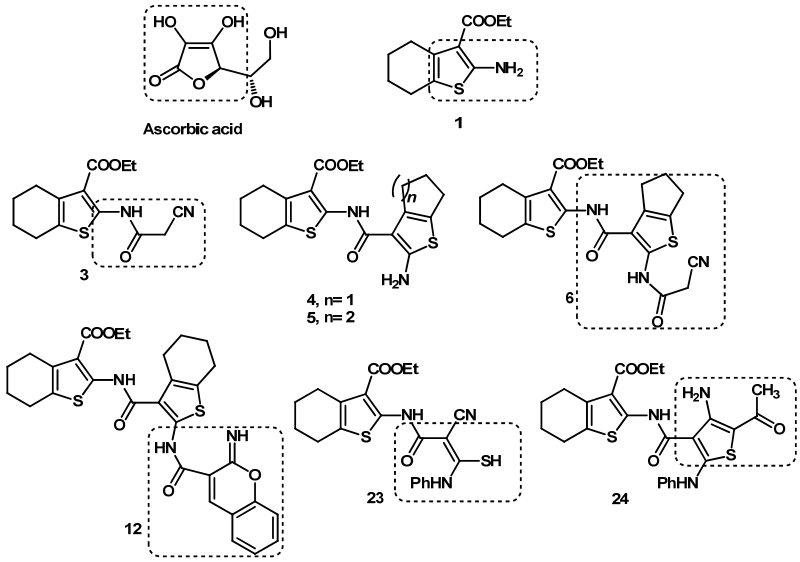


Figure 1. Structure activity relationship of the more potent antioxidant compounds.

to the previously reported method *via* refluxing of ethyl 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**1**) [17] with 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropane nitrile (**2**) [18].

Multicomponent condensation of cyclopentanone or cyclo hexanone with compound **3** and elemental sulfur containing morpholine as a catalyst afforded the bithiophene derivatives **4** and **5**, respectively. Compounds **4** and **5** form hydrogen bonds between carbonyl of esters and NH groups of amide and between amino groups and amidic groups. Because the IR spectra revealed low stretching vibrations of ester and amidic carbonyl groups and the ^{1}H NMR spectra show the presence of NH singlet signal at 11.04 and 11.10 ppm, respectively, similar behaviour was reported [21]. Moreover, cyanoacetylation of compound **4** and **5** under the same previous conditions afforded the cyanoacetamide derivatives **6** and **7**. Treatment of compound **7** with cyclopentanone or cyclohexanone and elemental sulfur in ethanol containing morpholine afforded the corresponding trithiophene derivatives **8** and **9**, respectively. Compound **8** and **9** formed three hydrogen bonds (Scheme 1).

Knoevenagel condensation of compound **7** with 4-*N*,*N*-dimethylaminobezaldehyde, 4-piperdin-1-ylbenzaldehyde or salicyaldehyde in ethanol containing a catalytic amount of piperidine afforded the corresponding (*E*) arylidenes **10**, **11** and coumarin derivative **12**, respectively [22] (Scheme 2).

Furthermore, condensation of compound **3** with terphthaldehyde in ethanol containing a catalytic amount of piperidine achieved the (*E*) *bis*-arylidene derivative **13** [23]. Coupling of compound **3** with benzene-1,4-*bis*(diazonium) dichloride afforded the (*E*) hydrazo derivative **14** [21] (Bioisostere of compound **13**) (Scheme 3).

On the other hand, stirring of compound **3** with carbon disulphide in DMF containing potassium hydroxide followed by in situ addition of ethyl bromoacetate afforded the triester derivative **16** *via* the intermediate **15**, which afforded the dithiophene **17** upon heating in DMF containing a catalytic amount of triethylamine (Scheme 4).

Attempting for preparation of compounds **21** and **22**, which were bioisostere of compound **16** *via* reaction of compound **3** with phenyl isothiocyanate in dry DMF in the presence of potassium hydroxide followed by addition of reaction with chloroacetone or ethyl bromoacetate was failed. Although both compound **21** and **22** were formed *in situ*, subsequently cyclized to the 2-{[(2*Z*)-2-cyano-2-(4-methyl-3-phenyl-1,3-thiazol-2(3H)-ylidene)acetyl]amino}-4,5,6,7-tetra hydro-1-benzothiophene-3-carboxylate (**19**) and ethyl 2-{[(2Z) -2-cyano-2-(4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene)acetyl] amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**20**), respectively [8]. Acidification of the intermediate potassium salt **15** afforded compound **23** [8], refluxing of compound **23** with chloroacetone or ethyl bromoacetate afforded the dithiophene derivatives **24** and **25** (Bioisoster of compound **17**), respectively (Scheme 5). The structure of new synthesized compounds was established on the basis of their elemental analyses and spectral data (IR, ^{1}H, ^{13}C NMR and mass spectral data, c.f. Experimental section).

3.2. Biological Assays

3.2.1. Superoxide anion radical scavenging assay

The antioxidant activity of the new synthesized compounds was evaluated following the protocols published by *Nishimiki et al*. [19]. The biological data showed clearly that compounds **1**, **7**, **8**, **12**, **16** and **23** have good activities, while compounds **3**-**6**, **9**, **10**, **14** and **17** exhibited moderate activities. On the other hand, the anther compounds exhibited weak activities (Table 1). Thus, it would appear that introducing of cyanoacetamide, thiocarbamoyl, thiophene and coumarin moieties enhances the antioxidant properties of 2-aminothiophene derivatives.

All compounds were dissolved in DMSO:MeOH (1:1, *v*:*v*) and tested at the final concentration of 0.1 mL of 1 mg/mL. The extent of DNA damage is expressed by increase of absorbance at 520 nm. The synthesized compounds were test for Bleomycin-dependent DNA damage (Table 1) and showed that compounds **1**, **4**, **5**, **12**, **23** and **24** have an ability to protect DNA from the induced damage by Bleomycin.

By comparing the results obtained of antioxidant of the compounds reported in this paper to their structures, the following structure activity relationship (SAR's) were postulated:

(i) 2-Aminothiophene derivatives **1**, **4** and **5** are more potent than ascorbic acid which may be attributed to the replacement of furan moiety with the thiophene moiety and presence of amino group.

(ii) Compounds **3**, **6** and **7** (*N*-substituted-2-amino thiophene) is less potent than compounds **1**, **4** and **5**,

respectively which may be due to decrease of basicity of amino group.

(iii) Sulfanyl derivative **23** is more potent than **3** 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) Compounds **12** and **24** exhibited high antioxidant activity which may be due presence of imino coumarin and 3-aminothiophene moiety (Figure 1).

4. Conclusion

The objective of the present study was to synthesize and evaluate the antioxidant activity of some novel *bis*-thiophenes with the hope of discovering new structure leads serving as antioxidant agents. The data clearly showed that compounds **1**, **7**, **8**, **12**, **16** and **23** have good activities exhibited weak anti-oxidant activities by Superoxide anion radical scavenging assay. On the other hand, compounds **1**, **4**, **5**, **12**, **23** and **24** have an ability to protect DNA from the induced damage by Bleomycin.

Acknowledgements

We deeply appreciate the assistance of Dr. Ahmed Abass, Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt for screening of the Biological activities of the tested compounds

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