Full Paper

Synthesis and Antioxidant Activity of a Novel Series of Pyrazolotriazine, Coumarin, Oxoazinone, and Pyrazinopyrimidine Derivatives

Moustafa A. Gouda[1,2]

[1] Faculty of Science, Department of Chemistry, Mansoura University, Mansoura, Egypt
[2] Faculty of Science and Arts, Department of Chemistry, Ulla, Taibah University, Medina, KSA

A series of pyrazolotriazone derivatives **8–10** were obtained via coupling of 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (**1**) with 3-pyrazole diazonium chlorides **2–4**, followed by heating of the formed hydrazones **5–7** in acetic acid, respectively. Moreover, coupling reaction of **1** with the aryl diazonium salts **15a–c** afforded hydrazones **16a–c**. Furthermore, treatment of **1** with 2-hydroxy-1-aldehydes **18–20** afforded the corresponding coumarins **21–23**, respectively. Finally, compound **1** reacted with 1-nitrosonaphthalen-2-ol (**26**) and 6-amino-5-nitroso-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**28**) to give 3-oxo-3*H*-naphtho[2,1-*b*][1,4]oxazine-2-carbonitrile (**25**) and pyridazinopyrimidine (**29**), respectively. The newly synthesized compounds were screened for their ABTS antioxidant activity. Compounds **7** (90.39%), **10** (85.88%), and **16a** (91.95%) exhibited promising activities. The most potent compound, **16a**, has the ability to protect DNA from the damage induced by bleomycin.

Keywords: ABTS antioxidant assay / Bleomycin-dependent DNA damage / Coumarin / Oxopropanenitrile / Pyrazolotriazone / Pyrimidine

Received: April 11, 2013; Revised: May 10, 2013; Accepted: May 24, 2013

DOI 10.1002/ardp.201300128

Introduction

Various substituted 1,2,4-triazin-5-one derivatives have great importance in the medicinal and agricultural fields [1–5]. Recently, significant biological activities of this class of compounds have been observed, in particular of 4-amino-1,2,4-triazin-5(2*H*)-one derivatives working as herbicidal [6, 7], antimicrobial [8–10], anti-HIV [11], and anticancer agents [12].

The piperazinone ring has largely been used as a rigid template in the construction of novel receptor ligands [13]. For example, these heterocyclic compounds are of great biological interest since they represent the structural core of several biologically active compounds, such as Leu-enkephalin analogs [14], cholecystokinin receptor antagonists [15], RGD mimetics [16, 17], and the neurokinin-2 receptor ligand [18].

Coumarin (2*H*-chromen-2-one) and its derivatives are widely distributed in nature and have been reported to exhibit diverse pharmacological properties such as anticancer [19], anti-coagulant, estrogenic, dermal, photosensitizing, antimicrobial, vasodilatory, molluscicidal, antihelminthic, sedative, hypnotic, analgesic [20, 21], hypothermic [20, 21], and free radical-scavenging activities, especially of the superoxide anions generated by activated neutrophils [22].

Furthermore, 1,4-benzoxazinone-based compounds have a wide range of biological activities and pharmaceutical actions, e.g., as inhibitors of bacterial histidine protein kinase [23, 24], in treating heart diseases [25], and as inhibitors of nitric oxide synthase (NOS), thus representing potential drugs for the treatment of neurodegenerative, inflammatory, autoimmune, and cardiovascular disorders [26, 27].

On the other hand, 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (**1**) is a very handy and cheap cyanoacetylation reagent, which was first synthesized and introduced in the late 1950s by Ried and Schleimer [28]. It was successfully

Correspondence: Dr. Moustafa A. Gouda, Faculty of Science, Department of Chemistry, Mansoura University, El-Gomhoria Street, Mansoura 35516, Egypt.
E-mail: dr_mostafa_chem@yahoo.com
Fax: +20 50 2246781

applied for the synthesis of various *N*-alkyl and *N*-aryl cyanoacetamides [29, 30]. Recently, Gorobets et al. [31] prepared a series of cyanoacetamides via refluxing of **1** with the appropriate amine in toluene. We recently reported the synthesis of 2-(2-cyanoacetylamino)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylic acid ethyl ester from compound **1** [32].

Herein, compound **1** was used as a key intermediate for the synthesis of coumarin, oxazine, pyrazolotriazinone, and pyrazinopyrimidine derivatives in high yield and purity, in order to investigate their antioxidant activities.

Results and discussion

Chemistry

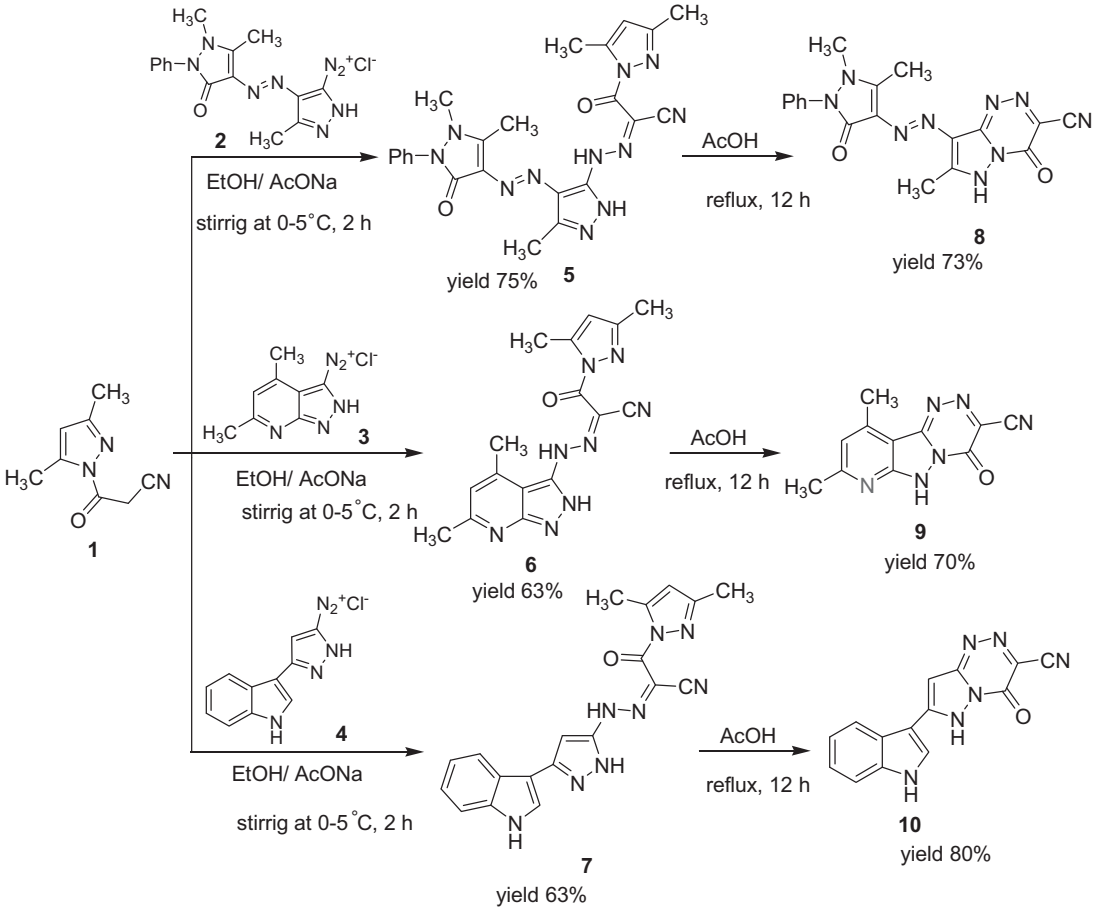
Compound **1** was used to synthesize the pyrazolotriazone derivatives **8–10** via coupling with the corresponding 3-pyrazole diazonium chlorides **2–4** [33–35], followed by heating the formed hydrazones **5–7** in acetic acid (Scheme 1).

An analogous reaction has been reported by El-Deen et al. [36], where as the 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]-pyridine-3-diazonium chloride **3** was coupled with ethyl cyanoacetate to give the corresponding hydrazono derivative **11**. Compound **11** gave the corresponding aminopyrazolotriazine derivative **12** upon refluxing in acetic acid and did not afford the pyrazolotriazinone **9** (Scheme 2).

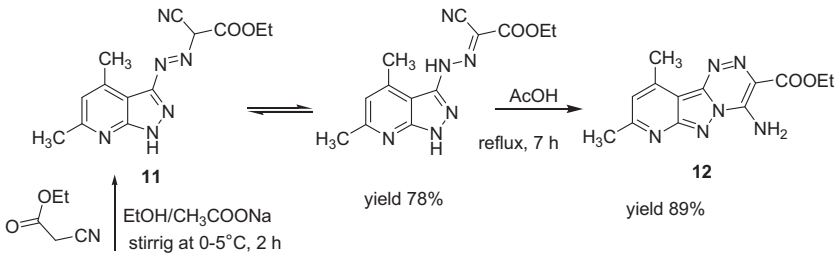
Furthermore, Metwally et al. [33] reported that coupling of the diazonium salt of 4-((3-amino-5-methyl-1*H*-pyrazol-4-yl)-diazenyl)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**2**) with ethyl cyanoacetate gave the corresponding hydrazono derivative **13**. Compound **13** gave the corresponding aminopyrazolotriazine derivative **14** upon refluxing in acetic acid and did not afford the pyrazolotriazinone **8** (Scheme 3).

The coupling of **1** with the corresponding aryl diazonium salts **15a–c** afforded the hydrazine derivatives **16a–c**. Attempts to prepare 4-oxo-3,4-dihydrocinnoline-3-carbonitrile derivatives **17a–c** (bioisosters of **8–10**) via cyclization of **16a–c** were unsuccessful (Scheme 4).

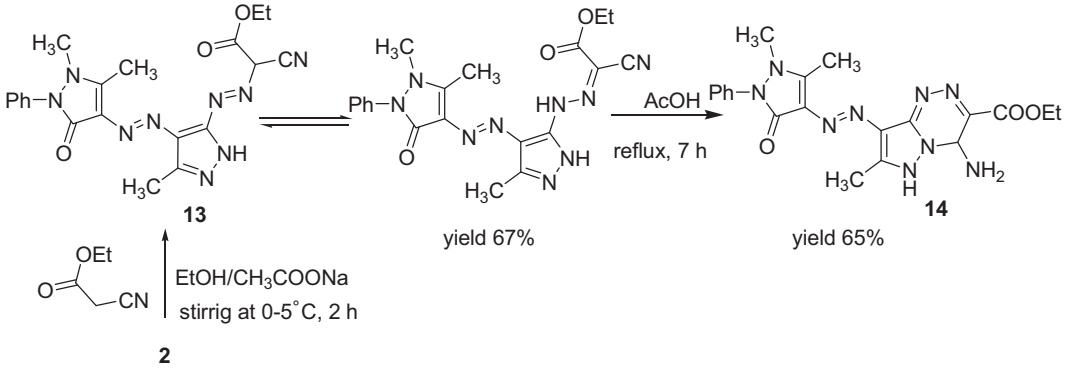
The reactivity of **1** towards 2-hydroxy carboxaldehydes was studied in order to prepare the corresponding coumarin derivatives in a one-pot reaction with high yields. Thus,



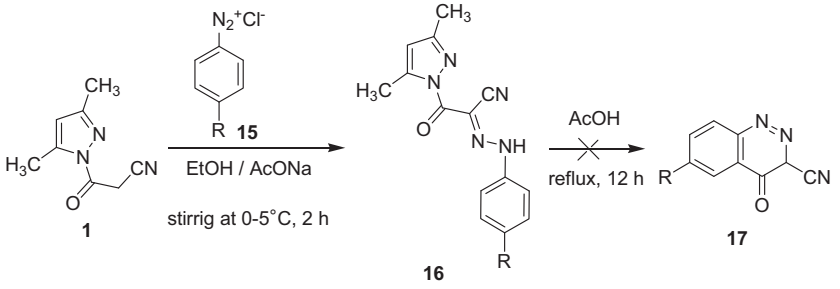
Scheme 1. Synthesis of the pyrazolotriazone derivatives **8–10**.



Scheme 2. Synthesis of the aminopyrazolotriazine derivative **12**.



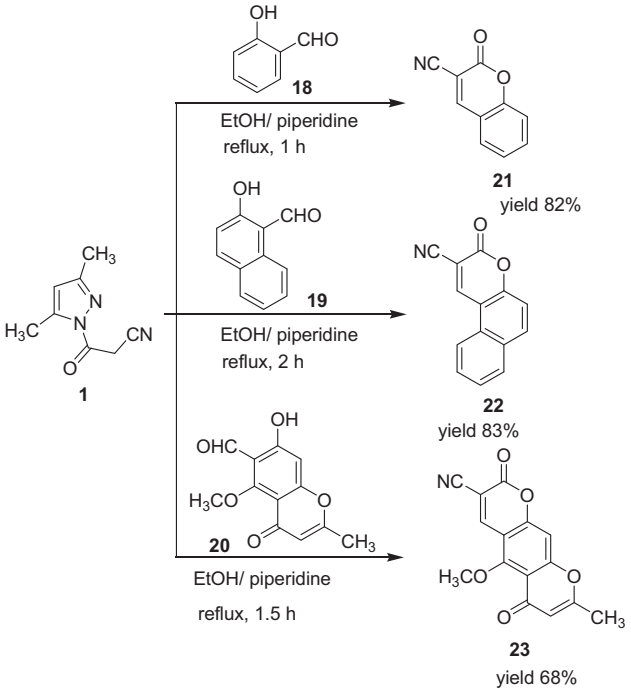
Scheme 3. Synthesis of the aminopyrazolotriazine derivative **14**.



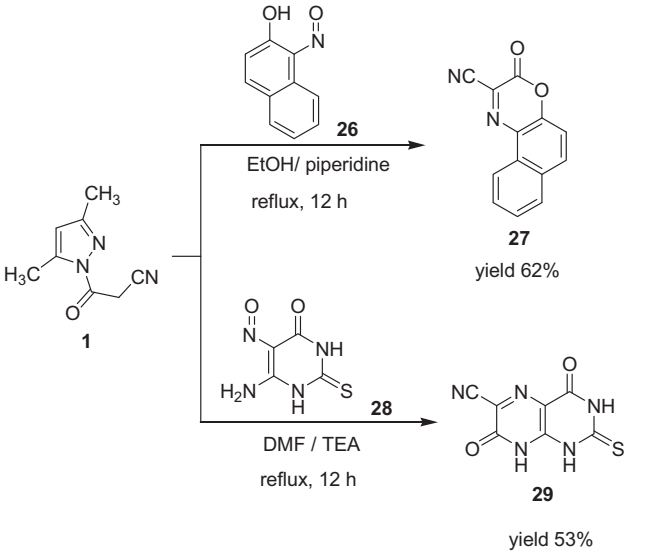
Scheme 4. Synthesis of the hydrazone derivatives **16a–c**.

treatment of **1** with salicylaldehyde (**18**), 2-hydroxy-1-naphthaldehyde (**19**) or 7-hydroxy-5-methoxy-2-methyl-4-oxo-4*H*-chromene-6-carboxaldehyde (**20**) afforded the corresponding coumarin derivatives **21**–**23** (Scheme 5). Yamashita et al. [37] prepared the coumarin derivative **21** in a multistep synthesis as outlined in Scheme 6 via Knoevenagel reaction of salicyladehyde with malononitrile using isopropyl alcohol as solvent in Ti(O-*i*-Pr)$_4$, followed by aqueous hydrolysis for the formed iminocoumarin **25**.

Furthermore, the synthesis of compound **27**, a bioisoster of **22**, was also investigated. Condensation of **1** with 1-nitrosonaphthalen-2-ol **26** in ethanol catalyzed by piperidine was carried out to obtain 3-oxo-3*H*-naphtho[2,1-*b*][1,4]oxazine-2-carbonitrile **27**. Moreover, the synthesis of compound **29**, a



Scheme 5. Synthesis of the coumarin derivatives **21**–**23**.



Scheme 7. Synthesis of naphtho[2,1-*b*][1,4]oxazine **27** and the pyridazinopyrimidine derivative **29**.

bioisoster of **17**, was also investigated. Thus, compound **1** reacted with 6-amino-5-nitroso-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**28**) in dimethylformamide (DMF) catalyzed by triethylamine in order to obtain the corresponding pyridazinopyrimidine derivative **29** (Scheme 7). The formation of **29** can be explained according to the postulated mechanism shown in Fig. 1.

Biological evaluation

ABTS antioxidant assay

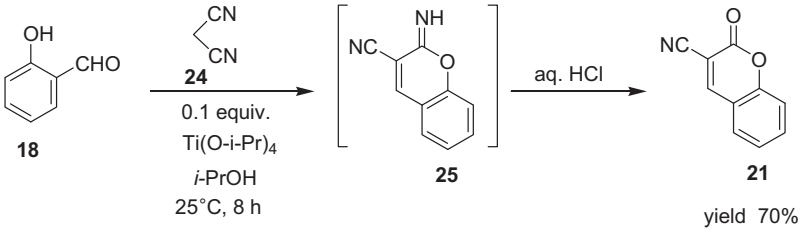
The newly synthesized compounds were tested for their antioxidant activity using the ABTS method as reported by Lissi et al. [38]. The antioxidant activity assay employed herein is one of several assays that depend on measuring the consumption of stable free radicals; that is, it evaluates the free radical-scavenging activity of the investigated component. The methodology assumes that the consumption of the stable free radical ($X^{\bullet}$) will be determined by the following reaction:

$$XH + Y^{\bullet} \rightarrow X^{\bullet} + YH$$

where XH is the tested compound, $Y^{\bullet}$ is the radical released from ABTS, and YH is scavenging the free radical $X^{\bullet}$.

The rate and/or the extent of the process is measured in terms of the decrease in $X^{\bullet}$ concentration, which is related to the ability of the added compounds to trap free radicals. The decrease in color intensity of the free radical solution due to scavenging of the free radical by the antioxidant material is measured calorimetrically at a specific wavelength (e.g., 734 nm). The assay employs the radical cation derived from 2,2′-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid; ABTS) as a stable free radical to assess the antioxidant potential of the investigated compounds.

Some of the compounds displayed antioxidant activity compared with ascorbic acid, as shown in Table 1. Compounds **7**, **10**, and **16a** displayed high antioxidant potency, while compounds **16b**, **21**, and **27** showed moderate



Scheme 6. Multistep synthesis of the coumarin derivative **21**.

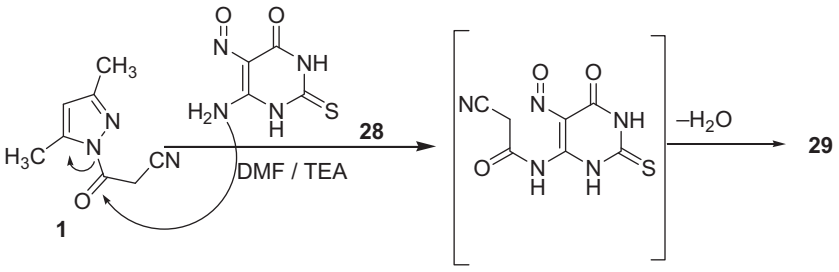


Figure 1. The postulated mechanism of formation of compound **29**.

antioxidant activity; the remaining compounds showed weak antioxidant activity. Compound **16a** exhibited the highest antioxidant activity comparable to that of ascorbic acid.

Bleomycin-dependent DNA damage assay

Bleomycins are a family of glycopeptide antibiotics that were used routinely as antitumor agents. The bleomycin assay was adopted for assessing the pro-oxidant effects of food antioxidants. The antitumor antibiotic bleomycin binds iron ions and DNA. The bleomycin–iron complex degrades DNA which, upon heating with thiobarbituric acid (TBA), yields a pink chromogen. Upon the addition of suitable reducing agents, antioxidants compete with the DNA and diminish chromogen formation [39]. The protective activity against DNA damage induced by the bleomycin–iron complex was examined in order to show the action of the more potent compounds **7**, **10**, **16a**, and **21**. The results in Table 2 show that compound **16a** exhibits high protection against DNA damage induced by the bleomycin–iron complex, thus diminishing chromogen formation between the damaged DNA and TBA molecules.

Table 1. ABTS antioxidant activity assay of the new compounds.

Compound no.	Absorbance of samples (λ)	% Inhibition
Control of ABTS[a)]	0.510	0%
Ascorbic acid	0.042	91.76%
5	0.439	13.92%
6	0.466	8.62%
7	0.049	90.39%
8	0.465	8.82%
9	0.483	5.29%
10	0.072	85.88%
16a	0.043	91.95%
16b	0.211	58.62%
16c	0.405	20.58%
21	0.168	67.05
22	0.488	4.31
23	0.434	14.90
27	0214	52.74
29	0.362	20.01

[a)] ABTS: The method used for antioxidant activity
(%) Inhibition $= [(A_{control} - A_{test})/A_{control}] \times 100$.

Table 2. Bleomycin-dependent DNA damage of the investigated compounds.

Compound no.	Absorbance of samples
Ascorbic acid	0.087
7	0.099
10	0.110
16a	0.085
21	0.091

Structure–activity relationships (SAR) of the tested compounds for antioxidant activity

Comparing the results obtained for the antioxidant properties of the compounds reported in this study with their structures, the following SAR are postulated (Fig. 2): (i) It seems that the hydrazone and pyrazole moieties within **5**–**7** play an important role in the antioxidant activity, as their elimination through cyclization of the hydrazone derivatives **5**–**7** into the triazine derivatives **8**–**10** leads to a decrease in activity. (ii) Compounds **7** and **10** are more potent than compounds **5**, **6**, **8**, and **9**, which may be due to the presence of the indole moiety. (iii) The antioxidant activities of compounds **16a**–**c** follow the order **16a** > **16b** > **16c**, which may be explained by the electron-donating character of the substituents in *para*-position to the hydrazone moiety in the following order: $OCH_3 > -CH_3 > -Cl$. (iv) Compound **21** exhibited better antioxidant activity than compounds **22** and **23**, which may be explained by the extra ring/substituent in **22** and **23** decreasing the activity. (v) Compound **27** has higher antioxidant activity than compound **22**, which may be attributed to the replacement of the naphthoxazine by the benzocoumarin ring system.

Conclusion

The objective of the present study was to synthesize and evaluate the antioxidant activities of some novel coumarin, oxazine,

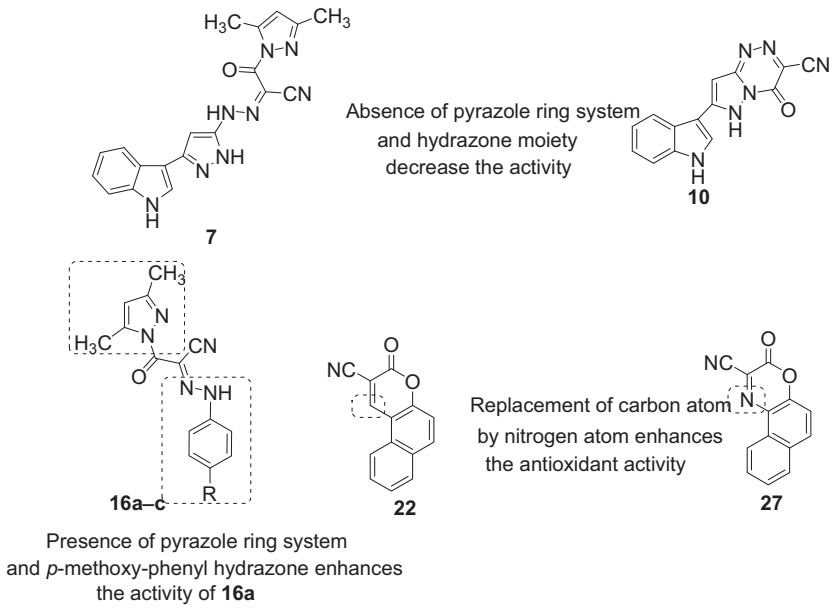


Figure 2. Structure–activity relationships of the more potent antioxidant compounds.

pyrazolotriazinone, and pyrazinopyrimidine derivatives, with the hope of discovering new structures serving as antioxidant agents. The data clearly showed that compounds **7**, **10**, **16a**, and **21** displayed promising *in vitro* antioxidant activities, by using the ABTS method. Also, compound **16a** exhibited high protection against the DNA damage induced by the bleomycin–iron complex, comparable to ascorbic acid.

Experimental

Chemistry

Instruments and methods

All melting points are given in degree Celsius (uncorrected) and were determined on a Gallenkamp electric melting point apparatus. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} precoated aluminum sheets. The IR spectra were recorded (KBr) on a Mattson 5000 FTIR spectrophotometer (λ, cm^{-1}) at the Microanalytical Unit, Faculty of Science, Mansoura University. The ^{1}H NMR spectra were carried out on a Varian spectrophotometer at 300 MHz using tetramethyl silane (TMS) as internal reference and DMSO-d_6 and chloroform as solvents; they were recorded at the Microanalytical Center, Cairo University. The mass spectra (EI) were recorded on a Varian Star 3400 Cx ion trap GC/MS Shimadzu GCMS-QP 5050 A EI (70 eV) at the Regional Center for Mycology and Biotechnology, Al-Azhar University. Elemental analyses (C, H, and N) were carried out at the Microanalytical Center, Cairo University, Giza, Egypt. The results of the elemental analysis were found to agree favorably with the calculated values. Biological activity determinations were carried out at the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

*General procedure for the synthesis of 3-(3,5-dimethyl-1H-pyrazol-1-yl)-2-arylhydrazono)-3-oxopropanenitrile derivatives **5**–**7***

A well-stirred solution of 4-((5-amino-3-methyl-1*H*-pyrazol-4-yl) diazenyl)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (1.60 g, 5 mmol), 3-amino-4,6-dimethyl-2*H*-pyrazolo[3,4-*b*]pyridine (0.81 g, 5 mmol) or 5-amino-3-(1*H*-indol-3-yl)-1*H*-pyrazole (0.99 g, 5 mmol) in a mixture of acetic acid (10 mL) and concentrated HCl (3 mL) was cooled in an ice bath, and then a solution of sodium nitrite (0.4 g, 5.8 mmol in 5 mL H_2O) was added dropwise under stirring. The above cooled diazonium solution was added slowly to a well-stirred solution of **1** (0.82 g, 5 mmol) in ethanol (25 mL) containing sodium acetate (2.4 g, 30 mmol). The reaction mixture was stirred for a further 2 h. The crude product was filtered-off, dried well, and crystallized from a mixture of EtOH/DMF to give compounds **5**, **6**, and **7**, respectively.

*3-(3,5-Dimethyl-1H-pyrazol-1-yl)-3-oxo-2-[2-{4-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-diazenyl)-5-methyl-1H-pyrazol-3-yl}hydrazono]-propanenitrile (**5**)*

Yield (1.82 g, 75%); orange powder; m.p. 212°C; IR (KBr): ν/cm = 3442 (OH), 2242 (CN), 1743, 1643 (2CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.58 (s, 3H, CH_3, antipyrine), 2.66 (s, 3H, CH_3 pyrazole), 2.93 (s, 3H, N–CH_3 antipyrine), 3.31 (br, 6H, 2CH_3), 3.94 (s, 1H, CH), 7.35–7.61 (m, 5H, Ar–H), 13.41 (s, 1H, OH); MS (EI, 70 eV) m/z (%) = 485 (3.3), 298 (3.6), 260 (4.0), 238 (5.7), 209 (4.2), 193 (6.2), 178 (8.9), 149 (7.3), 122 (33.2), 111 (24.2), 98 (17.1), 78 (25.5), 66 (46.7), 54 (100). Anal. calcd. for $C_{23}H_{23}N_{11}O_2$ (485.2): C, 56.90; H, 4.77; N, 31.73%. Found: C, 56.88; H, 4.72; N, 31.68%.

*3-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-(2-(4,6-dimethyl-2H-pyrazolo[3,4-b]pyridin-3-yl)hydrazono)-3-oxopropanenitrile (**6**)*

Yield (1.05 g, 63%); yellow powder; m.p. >320°C; IR (KBr): ν/cm = 3310, 3172 (2NH), 2221 (CN), 1683 (CO), 1633 (C=N), 1529 (N=N); MS (EI, 70 eV) m/z (%) = 335 (M^+−H, 0.2), 240 (26.1), 212 (16.0), 160 (21.2), 147 (21.3), 131 (36.0), 118 (21.0), 104 (19.6), 86 (71.7), 77 (22.0), 67 (14.0), 56 (100). Anal. calcd. for $C_{16}H_{16}N_8O$ (336.14): C, 57.13; H, 4.79; N, 33.31%. Found: C, 57.10; H, 4.75; N, 33.25%.

*2-(2-(3-(1H-Indol-3-yl)-1H-pyrazol-5-yl)hydrazono)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (**7**)*

Yield (1.17 g, 63%); pale green powder; m.p. 232°C; IR (KBr): ν/cm = 3244, 3192 (2NH), 2240 (CN), 1640 (CO), 1522 (N=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.37 (br, 6H, $2CH_3$); 4.48 (s, 1H, CH); 7.22–8.37 (m, 5H, Ar–H, NH-indole), 12.21 (br, s, 1H, NH-pyrazole), 12.28 (br, s, 1H, NH-hydrazone); MS (EI, 70 eV) m/z (%) = 261 [M^+−H+(3,5-dimethyl-1*H*-pyrazole-1-carbonyl), 0.2], 244 (0.3), 213 (2.4) 184, (10.6), 144 (100), 116 (32.9), 88 (42.5), 62 (31.3). Anal. calcd. for $C_{19}H_{16}N_8O$ (372.14): C, 61.28; H, 4.33; N, 30.09%. Found: C, 57.10; H, 4.75; N, 33.25%.

*General procedure for the synthesis of pyrazolotriazines **8**, **9**, and **10***

A suspension of arylazo derivatives **5** (2.42 g, 5 mmol), **6** (1.68 g, 5 mmol), and **7** (1.86 g, 5 mmol) in acetic acid (10 mL) was refluxed for 12 h. The reaction mixture was poured into ice-cold water and then neutralized with sodium bicarbonate. The formed precipitate was filtered-off, dried, and crystallized from a mixture of DMF/ethanol to give compounds **8**, **9**, and **10**, respectively.

*8-((2,3-Dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-4-yl)diazenyl)-7-methyl-4-oxo-4,6-dihydropyrazolo[5,1-c]-[1,2,4]triazine-3-carbonitrile (**8**)*

Yield (1.41 g, 73%); orange powder; m.p. 236°C; IR (KBr): ν/cm = 3442 (br, NH), 2242 (CN), 1743, 1643 (2CO), 1488 (N=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.57 (s, 3H, CH_3-antipyrine), 2.67 (s, 3H, CH_3-pyrazole), 3.13 (s, 3H, N–CH_3), 7.40–7.60 (m, 5H, Ar–H), 12.42 (s, 1H, NH); MS (EI, 70 eV) m/z (%) = 389 (2.0), 343 (1.4), 280, (1.0), 252 (6.3), 233 (5.4), 208 (1.4), 178 (5.8), 149 (14.8), 122 (11.8), 111 (18.2), 95 (24.6), 76 (41.0), 70 (52.1), 56 (100). Anal. calcd. for $C_{11}H_8N_6O$ (240.08): C, 55.52; H, 3.88; N, 32.38%. Found: C, 55.48; H, 3.81; N, 32.40%.

*3,4,8,9,9a-Pentaza-5,7-dimethyl-1-oxo-9,9a-dihydro-1H-fluorene-2-carbonitrile or pyrido[2,3:3,4]-4-oxo-4,6-dihydropyrazolo[5,1-c][1,2,4]triazine-3-carbonitrile (**9**)*

Yield (0.84 g, 70%); orange powder; m.p. >320°C; IR (KBr): ν/cm = 3444 (NH), 2253 (CN), 1727 (CO), 1639 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.70 (s, 3H, CH_3), 2.94 (s, 3H, CH_3), 3.37 (s, 1H, CH), 7.17 (s, 1H, Ar–H), 12.50 (s, 1H, NH); MS (EI, 70 eV) m/z (%) = 240 (10.4), 212 (8.0), 172 (11.5), 146 (24.9), 131 (31.1), 119 (38.2), 104 (67.9), 85 (18.6), 76 (68.9), 65 (68.4), 56 (100). Anal. calcd. for $C_{11}H_8N_6O$ (240.08): C, 55.00; H, 3.36; N, 34.98%. Found: C, 55.05; H, 3.31; N, 34.95%.

*4,6-Dihydro-7-(1H-indol-3-yl)-4-oxopyrazolo[5,1-c][1,2,4]-triazine-3-carbonitrile (**10**)*

Yield (1.10 g, 80%); pale green powder; m.p. 240°C; IR (KBr): ν/cm = 3245 (br, 2NH), 2241 (CN), 1640 (CO); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 1H, CH), 7.09–8.35 (m, 5H, Ar–H), 12.26 (s, 1H, NH), 14.62 (s, 1H, NH); MS (EI, 70 eV) m/z (%) = 278 (2.0), 231 (0.2), 184 (21.0), 144 (100), 116 (40.7), 88 (31.9), 62 (26.0), 50 (16.8). Anal. calcd. for $C_{14}H_8N_6O$ (276.08): C, 60.87; H, 2.92; N, 30.42%. Found: C, 60.84; H, 2.88; N, 30.40%.

*General procedure for the synthesis of 3-(3,5-dimethyl-1H-pyrazol-1-yl)-2-arylhydrazono)-3-oxopropanenitrile derivatives **16a–c***

A well-stirred solution of 4-anisidine (0.62 g, 5 mmol), *p*-toluidine (0.54 g, 5 mmol) or 4-chloroaniline (0.64 g, 5 mmol) in concentrated HCl (3 mL) was cooled in an ice bath, and then a solution of sodium nitrite (0.4 g, 5.8 mmol in 5 mL H_2O) was added dropwise. The above cooled diazonium solution was added slowly to a well-stirred solution of **1** (0.82 g, 5 mmol) in ethanol (25 mL) containing sodium acetate (2.4 g 30 mmol). The reaction mixture was stirred for a further 2 h. The obtained crude product was filtered-off, dried well, and crystallized from ethanol to give compounds **16a–c**, respectively.

*2-(2-(4-Methoxyphenyl)hydrazono)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (**16a**)*

Yield (1.26 g, 85%); orange powder; m.p. 142°C; IR (KBr): ν/cm = 3160 (NH), 2224 (CN), 1684 (CO), 1610 (C=N), 1512 (N=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.35 (br, 6H, $2CH_3$), 3.80 (s, 3H, OCH_3), 6.95–7.48 (m, 5H, Ar–H, $CH_{pyrazole}$), 12.19 (s, 1H, NH); MS (EI, 70 eV) m/z (%) = 298 (M^++1, 0.7), 251 (5.5), 233 (28.4), 178 (34.3), 152 (12.4), 136 (26.0), 121 (84.9), 106 (29.4), 91 (28.4), 77 (50.7), 63 (65.7), 51 (100). Anal. calcd. for $C_{15}H_{15}N_5O_2$ (297.31): C, 60.60; H, 5.09; N, 23.56%. Found: C, 60.66; H, 5.05; N, 23.60%.

*2-(2-(4-Tolyl)hydrazono)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (**16b**)*

Yield (1.17 g, 83%); red powder; m.p. 129°C; IR (KBr): ν/cm = 3167 (NH), 2222 (CN), 1683 (CO), 1593 (C=C), 1534 (N=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.28 (s, 3H, CH_3), 3.32 (br, 6H, $2CH_3$), 7.20–7.42 (m, 5H, Ar–H, $CH_{pyrazole}$), 12.19 (s, 1H, NH); MS (EI, 70 eV) m/z (%) = 281 (M^+, 0.6), 251 (91.9), 217 (57.5), 208 (33.7), 179 (10.0), 157 (28.9), 125 (6.8), 105 (87.1), 91 (45.1), 76 (100), 65 (38.1), 50 (82.3). Anal. calcd. for $C_{15}H_{15}N_5O$ (281.31): C, 64.04; H, 5.37; N, 24.90%. Found: C, 64.06; H, 5.35; N, 24.85%.

*2-(2-(4-Chlorophenyl)hydrazono)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (**16c**)*

Yield (1.13 g, 75%); reddish brown powder; m.p. 176°C; IR (KBr): ν/cm = 3180 (NH), 2224 (CN), 1686 (CO), 1591 (C=C), 1526 (N=N), 780 (C–Cl; MS (EI, 70 eV) m/z (%) = 301 (M^+, 0.2), 237 (19.7), 177 (34.6), 125 (73.0), 111 (46.8), 99 (70.4), 89 (52.5), 74 (100), 62 (38.1), 50 (42.6). Anal. calcd. for $C_{14}H_{12}ClN_5O$ (301.73): C, 55.73; H, 4.01; N, 23.21%. Found: C, 55.66; H, 4.05; N, 23.15%.

*General procedure for the synthesis of coumarin derivatives **21–23** and oxazinone **27***

A suspension of **1** (0.82 g, 5 mmol) and 2-hydroxybenzaldehyde **18** (0.61 g, 5 mmol), 2-hydroxy-1-naphthaldehyde **19** (0.86 g, 5 mmol), or 7-hydroxy-5-methoxy-2-methyl4-oxo-4*H*-chromene-6-carbaldehyde **20** (1.17 g, 5 mmol) or 1-nitroso naphthalen-2-ol **26** (0.86 g, 5 mmol) in ethanol (25 mL) containing piperidine (0.42 g, 6 mmol) was refluxed for the appropriate time. The reaction mixture was poured into ice-cold water; then, the formed precipitate was filtered-off, dried, and recrystallized from the proper solvent to give compounds **21**–**23** and **27**, respectively.

*3-Oxo-2H-chromene-3-carbonitrile (**21**)*

Yield (0.70 g, 82%); reaction time 1 h, crystallization from ethanol; yellow powder; m.p. 187°C [lit. 184°C]; IR (KBr): ν/cm = 2207 (CN), 1740 (CO), 1610 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.02–8.25 (m, 4H, Ar–H), 9.44 (s, 1H, C_4-H); MS (EI, 70 eV) m/z (%) = 172 (M^+, 0.2), 154 (4.1), 143 (0.9), 127 (1.6), 115 (9.4), 101 (8.4), 88 (100.0), 62 (35.6), 50 (37.3). Anal. calcd. for $C_{10}H_5NO_2$ (171.03): C, 70.18; H, 2.94; N, 8.18%. Found: C, 70.13; H, 2.95; N, 8.13%.

*3-Oxo-3H-benzo[f]-chromene-3-carbonitrile (**22**)*

Yield (0.92 g, 83%); reaction time 2 h, crystallization from ethanol/benzene; yellow powder; m.p. 303°C; IR (KBr): ν/cm = 2226 (CN), 1731 (CO), 1601 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.63–8.65 (m, 6H, Ar–H), 9.78 (s, 1H, C_1-H); MS (EI, 70 eV) m/z (%) = 221 (M^+, 100), 193 (66.8), 164 (58.1), 138 (28.9), 113 (9.7), 96 (7.9), 87 (147), 74 (9.9), 62 (17.2). Anal. calcd. for $C_{14}H_7NO_2$ (221.21): C, 76.01; H, 3.19; N, 6.33%. Found: C, 76.05; H, 3.15; N, 6.30%.

*5-Methoxy-8-methyl-2,6-dioxo-6,9-dihydro-2H-pyrano-[2,3-g]chromene-3-carbonitrile (**23**)*

Yield (0.96 g, 68%); reaction time 1.5 h, crystallization from ethanol/benzene; pale yellow powder; m.p. 273°C; IR (KBr): ν/cm = 2219 (CN), 1776, 1664 (2CO), 1615 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.35 (s, 3H, CH_3), 3.96 (s, 3H, OCH_3), 6.19 (s, 1H, CH), 7.39 (s, 1H, CH), 8.67 (s, 1H, CH); MS (EI, 70 eV) m/z (%) = 284 (M^++1, 25.9), 257 (31.4), 242 (14.0), 228 (100), 200 (49.4), 172 (41.5), 157 (21.7), 143 (29.0), 114 (30.7), 101 (38.4), 86 (38.4), 68 (1.6), 58 (5.5), 62 (17.2). Anal. calcd. for $C_{15}H_9NO_5$ (283.05): C, 63.61; H, 3.20; N, 4.94%. Found: C, 63.60; H, 3.15; N, 4.93%.

*3-Oxo-3H-naphtho[2,1-b[1,4]]oxazine-3-carbonitrile (**27**)*

Yield (0.69 g 62%); reaction time 12 h, crystallization from ethanol/benzene; black powder; m.p. >315°C; IR (KBr): ν/cm = 2201 (CN), 1726 (CO), 1621 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.45–7.94 (m, 6H, Ar–H,); MS (EI, 70 eV) m/z (%) = 222 (M^+, 1.5), 208 (20.4), 164 (100), 151 (39.5), 143 (13.1), 129 (24.2), 115 (16.1), 97 (12.3), 77 (19.4), 69 (29.5), 55 (81.9). Anal. calcd. for $C_{13}H_6N_2O_2$ (222.04): C, 70.27; H, 2.72; N, 12.61%. Found: C, 70.23; H, 2.69; N, 12.66%.

*4,7-Dioxo-2-thioxo-1,2,3,4,7,8-hexahydropteridine-6-carbonitrile (**29**)*

A suspension of **1** (0.82 g, 5 mmol) and 6-amino-5-nitroso-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **28** (0.86 g, 5 mmol) in DMF (25 mL) containing triethylamine (0.6 g, 6 mmol) was refluxed for 12 h. The reaction mixture was poured into ice-cold water; then, the formed precipitate was filtered-off, dried, and crystallized from the DMF/EtOH solvent to give compound **29**.

Yield (0.52 g, 53%); washing from hot DMF; brown powder >315°C; IR (KBr): ν/cm = 3300–3190 (br, 3NH), 2193 (CN), 1715, 1651 (2CO), 1610 (C=N); MS (EI, 70 eV) m/z (%) = 221 (M^+, 0.5), 216 (6.1), 178 (23.6), 151 (15.4), 136 (26.9), 119 (39.1), 105 (15.2), 90 (40.9), 77 (61.1), 63 (69.6), 54 (100). Anal. calcd. for $C_7H_3N_5O_2S$ (221.2): C, 38.01; H, 1.37; N, 31.66%. Found: C, 37.99; H, 1.32; N, 31.61%.

Pharmacology

ABTS screening assay [38]

Antioxidant activities were evaluated from the bleaching of ABTS-derived radical cations. The radical cations derived from ABTS (2,2′-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid) were prepared by reaction of ABTS (60 mL) with MnO_2 (3 mL, 25 mg/mL) in sodium phosphate buffer (5 mL, 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 mL of a 1 mg/mL 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:

$$(\%)\text{Inhibition} = \left[\frac{A_{control} - A_{test}}{A_{control}}\right] \times 100$$

Ascorbic acid (20 mL, 2 mM) solution was used as a standard antioxidant (positive control). Blank samples were run using solvent without ABTS (Table 1).

Bleomycin-dependent DNA damage assay [40, 41]

To the reaction mixtures in a final volume of 1.0 mL, the following reagents were added at the final concentrations stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), magnesium chloride (5 mM), KH_2PO_4/KOH buffer pH 7.0 (30 mM), and ascorbic acid (0.24 mM), and the test fractions diluted in MeOH to give a concentration of (0.1 mg/mL). The reaction mixtures were incubated in a water bath at 37°C for 1 h. At the end of the incubation period, 0.1 mL ethylenediaminetetraacetic acid (EDTA; 0.1 M) was added to stop the reaction (the iron–EDTA complex is unreactive in the bleomycin assay). DNA damage was assessed by adding 1 mL 1% w/v TBA and 1 mL 25% v/v hydrochloric acid (HCl), followed by heating in a water bath maintained at 80°C for 15 min. The chromogen formed was extracted into 1-butanol, and the absorbance was measured at 532 nm.

This work was supported by Taibah University, Medina, KSA, project No. 433/1921, year: 2012.

The author has declared no conflict of interest.

References

[1] B. S. Holla, B. K. Sarojini, R. Gonsalves, *Farmaco* **1998**, *53*, 395–398.

[2] B. S. Holla, B. K. Sarojini, K. Shridhara, G. Antong, *Farmaco* **1999**, *54*, 149–151.

[3] A. Deeb, F. El-Mariah, M. Hosny, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5013–5017.

[4] M. A. El-Badawi, A. A. El-Barbary, Y. M. Loksha, M. El-Daly, *Phosphorus, Sulfur Silicon Relat. Elem.* **2002**, *177*, 587–596.

[5] A. S. Oganisyan, G. O. Grigoryan, A. S. Noravyan, I. A. Dzhagatspanyan, G. G. Melikyan, *Pharm. Chem. J.* **2001**, *35*, 124–126.

[6] W. Draber, C. Fedtke, *Advances in Pesticide Science* (Ed: H. Geissbtihler), Part 3, Pergamon Press, Oxford **1978**, p. 475.

[7] M. Timer, S. Sauvard, C. M. Georgescu, *Biochem. Pharmacol.* **1996**, *15*, 408–410.

[8] K. Singh, M. S. Barwa, P. Tyagi, *Eur. J. Med. Chem.* **2007**, *42*, 394–402.

[9] T. E. Ali, *Phosphorus, Sulfur Silicon Relat. Elem.* **2007**, *182*, 1717–1726.

[10] D. J. Prasad, M. S. Karthikeyan, P. B. Karegoudar, B. Poojary, B. S. Holla, N. S. Kumari, *Phosphorus, Sulfur Silicon Relat. Elem.* **2007**, *182*, 1083–1091.

[11] A. A. El-Barbary, A. M. Sakran, A. M. El-Madani, N. Claus, *J. Heterocycl. Chem.* **2005**, *42*, 935–941.

[12] Z. El-Gendy, J. M. Morsy, H. A. Allimony, W. R. Abdel-Monem, R. M. Abdel-Rahman, *Pharmazie* **2001**, *56*, 376–383.

[13] C. J. Dinsmore, D. C. Beshore, *Org. Prep. Proced. Int.* **2002**, *34*, 367–404.

[14] J. DiMaio, B. J. Belleau, *Chem. Soc., Perkin Trans.* **1989**, *1*, 1687–1689.

[15] D. A. Kendrick, H. Ryder, G. Semple, M. Szelke, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 9–12.

[16] B. C. Askew, C. J. McIntyre, C. A. Hunt, D. A. Claremon, R. J. Gould, R. J. Lynch, D. J. Armstrong, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 475–480.

[17] B. C. Askew, C. J. McIntyre, C. A. Hunt, D. A. Claremon, J. J. Baldwin, P. S. Anderson, R. J. Gould, R. J. Lynch, C. C.-T. Chang, J. J. Cook, J. J. Lynch, M. A. Holahan, G. R. Sitko, M. T. Stranieri, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1531–1536.

[18] M. E. Logan, R. Goswami, B. E. Tomczuk, B. R. Venepalli, *Annu. Rep. Med. Chem.* **1989**, *26*, 43–51.

[19] R. D. H. Murray, J. Mendez, S. A. Brown, *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*, John Wiley, New York **1982**, pp. 28–29.

[20] T. O. Soine, *J. Pharm. Sci.* **1964**, *53*, 231.

[21] R. O'Kennedy, R. D. Thornes (Ed.) *Coumarins E Biology, Applications and Mode of Action*, John Wiley, Chichester **1997**, pp. 125–141.

[22] M. Paya, P. A. Goodwin, B. De Las Heras, R. S. Hoult, *Biochem. Pharmacol.* **1994**, *48*, 445–451.

[23] M. Beach, R. Frechette, WO Patent Appl. 9728167, **1997**.

[24] R. Frechette, M. A. Weidner-Wells, WO Patent Appl. 9717333, **1997**.

[25] H. Tsutsui, M. Hori, I. Watanabe, K. Harada, J. Maruo, T. Morita, H. Ohtaka, T. Yamamoto, EP Patent Appl. 719766, **1996**.

[26] P. Hoelscher, R. Jautelat, H. Rehwinkel, S. Jaroch, D. Suelzle, M. Hillmann, G. A. Burton, F. M. McDonald, *Patent Appl.* WO0181324, **2001**.

[27] G. A. Burton, H. Rehwinkel, S. Jaroch, P. Hoelscher, D. Suelzle, M. Hillmann, F. M. McDonald, WO Patent Appl. 0017173, **2000**.

[28] a) W. Ried, B. Schleimer, *Angew. Chem.* **1958**, *70*, 164–169; b) *Chem. Abstr.* **1959**, *53*, 1314f.

[29] J. Stetinova, R. Kada, J. Lesko, *Molecules* **1996**, *1*, 251–254.

[30] J. Stetinova, R. Kada, J. Lesko, L. Zalibera, D. Ilavsky, A. Bartovic, *Collect. Czech. Chem. Commun.* **1996**, *61*, 921–929.

[31] N. Y. Gorobets, B. H. Yousefi, F. Belaj, C. O. Kappe, *Tetrahedron* **2004**, *60*, 8633–8644.

[32] M. A. Gouda, A. A. Abu-Hashem, *Arch. Pharm. Chem. Life Sci.* **2011**, *11*, 170–177.

[33] M. A. Metwally, M. A. Gouda, A. N. Harmal, A. M. Khalil, *Eur. J. Med. Chem.* **2012**, *56*, 254–262.

[34] a) M. Lacan, K. Tabakovic, *Croat. Chem. Acta* **1975**, *47*, 127–133; b) *Chem. Abstr.* **1976**, *84*, 59320s.

[35] H. M. Hassaneen, H. M. E. Hassaneen, Z. A. Gomaa, *Int. J. Org. Chem.* **2011**, *1*, 97–104.

[36] a) A. M. El-Dean, A. A. Geies, T. A. Mohamed, A. A. Atalla, *Bull. Fac. Sci. Assiut Univ.* **1991**, *20*, 15–21; b) *Chem. Abstr.* **1992**, *116*, 106161g.

[37] K. Yamashita, T. Tanaka, M. Hayashi, *Tetrahedron* **2005**, *61*, 7981–7985.

[38] E. A. Lissi, B. Modak, R. Torres, J. Esocbar, A. Urzua, *Free Radic. Res.* **1999**, *30* (6), 471–477.

[39] J. Gutteridge, D. Rowley, B. Halliwell, *Biochem. J.* **1981**, *199*, 263–265.

[40] B. F. Abdel-Wahab, A. S. El-Ahl, F. A. Badria, *Chem. Pharm. Bull.* **2009**, *57*, 1348–1351.

[41] F. A. Badria, M. Ameen, M. Akl, *Z. Naturforsch.* **2007**, *62c*, 656–660.

