Full Paper

Synthesis and Antioxidant Evaluation of Some New Pyrazolopyridine Derivatives

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4,6-Dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine (**1**) was used as a key intermediate for the synthesis of imidazolopyrazole derivatives **7–11** upon interaction with 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**), 2-(benzothiazol-2-yl)-4-chloro-3-oxobutanenitrile (**3**), 2,3-dibromonaphthalene-1,4-dione (**4**), naphtha[2,3-*b*]oxirene-2,7-dione (**5**), 2,5-dichloro-3,6-dihydroxyhexa-2,5-diene-1,4-dione (**6**), respectively. Acetylation of **11** afforded the *bis*-acetyl **12**. Also, the imidazolopyrimidine **15** was prepared *via* treatment of **1** with sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate (**13**) in DMF followed by cyclization of the *bis*-pyrazolopyrimidine **14** with glacial acetic acid. On the other hand, compound **1** was reacted with (*E*)-1-(4-methoxyphenyl)-5-(piperidin-1-yl)pent-1-en-3-one hydrochloride (**16**), 2-hydroxy-3-((piperidin-1-yl)-methyl)-naphthalene-1,4-dione (**17**), 2-styryl-2*H*-indene-1,3-dione (**18**), enaminone **22**, chloroquinoline-3-carbaldehyde **27a**, chloroquinoline-(6-methyl)-3-carbaldehyde **27b** and 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**28**) to afford pyrazolo[3,4-*a*]pyrimidines **19–21**, **23**, **29a**, **29b** and **30**, respectively. Also, the pyrazolopyrimidinone **33** was obtained *via* treatment of **1** with 1-cyanoacetyl-3,5-dimethylpyrazole (**31**) followed by cyclization of the formed intermediate **32** with glacial acetic acid. Finally, treatment of **1** with *o*-terephthalaldehyde in glacial acetic acid afforded diazepine **34**. The newly synthesized compounds were screened for their antioxidant properties in which some of them exhibited promising activities. Compounds **1**, **14**, **15**, **23**, **26**, **29a**, **30** and **32** have the ability to protect DNA from the damage induced by bleomycin.

Keywords: Antioxidant activity / Imidazolopyrazole / Pyrazolopyridine / Pyrazolopyrimidine

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Introduction

Pyrazole derivatives have attracted continued interest over the years due to use of this ring system as an important core structure in many drug substances, having a wide range of pharmacological applications [1–6]. Furthermore, the use of the pyrazolopyridine nucleus has pronounced pharmacological applications in anxiolytic [7], antiviral [8, 9], antileishmanial [10] and anti-inflammatory agents [11]. In continuation of our efforts [12–15] to identify new candidates that may be of value in designing new, potent, selective and less toxic antioxidant agents, we report herein the synthesis of some new fused heterocycles containing pyrazolopyridine systems starting from 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine (**1**).

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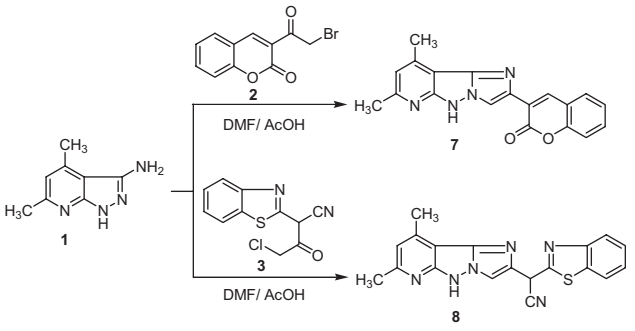
Results and discussion

Chemistry

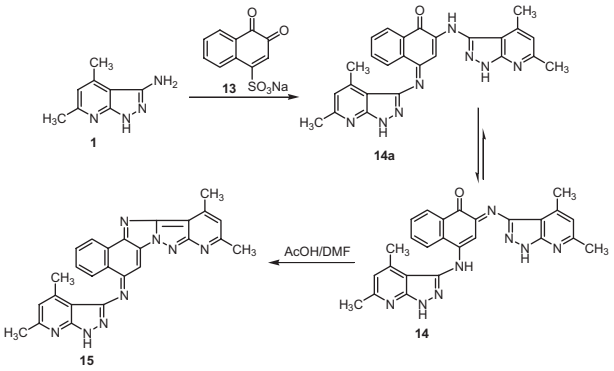
The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–6. Compound **1** was prepared according to the previously reported method [16].

Condensation of **1** with 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**) [17], 2-(benzothiazol-2-yl)-4-chloro-3-oxobutanenitrile (**3**) [18], 2,3-dibromonaphthalene-1,4-dione (**4**) [19], naphtha[2,3-*b*]oxirene-2,7-dione (**5**) [20] or 2,5-dichloro-3,6-dihydroxyhexa-2,5-diene-1,4-dione (**6**) in DMF/AcOH afforded the corresponding imidazo[1,2-*b*]pyrazole derivatives **7–11**, respectively. Acetylation of **11** with acetic anhydride afforded the *bis*-acetyl derivative **12** (Schemes 1 and 2).

Furthermore, the reactivity of aminopyrazole **1** towards sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulfonate (**13**) with the aim of preparing imidazo[1,2-*b*]pyrazole **15** was investigated. Thus, treatment of **1** with two equivalent



Scheme 1. Reactions of **1** with α-halo ketones.



Scheme 3. Reaction of **1** with 1,2-naphthoquinone derivative **13** followed by cyclocondensation.

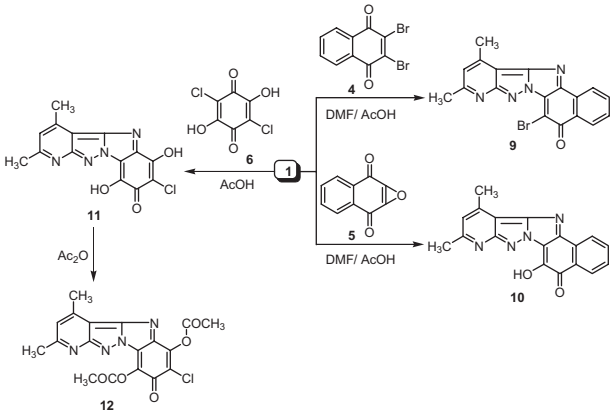
amounts of sodium 3,4-dihydro-3,4-dioxonaphthalene-1-sulfonate (**13**) through modification of the previously reported method for the synthesis of analogs [21] afforded the *bis*-pyrazolopyridine which upon treatment with acetic acid under reflux afforded the imidazopyrazole **15** (Scheme 3).

Pyrazolo[3,4-*a*]pyrimidines are of considerable chemical and pharmacological importance as purine analogues [22, 23]. Various related compounds of these also have anti-tumor, anti-leukemic activities. Several biological activities have been established for some pyrazolo derivatives [24, 25]. Also, the insecticidal activities have been investigated for some pyrazolo derivatives [26, 27]. We report herein the synthesis of some pyrazolo[1,5-*a*]pyrimidines from 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine (**1**). Thus, compound **1** was reacted with (*E*)-1-(4-methoxyphenyl)-5-(piperidin-1-yl)-pent-1-en-3-one hydrochloride (**16**) [28], 2-hydroxy-3-((piperidin-1-yl)-methyl)-naphthalene-1,4-dione (**17**) [29] and 2-styryl-2*H*-indene-1,3-dione (**18**) [30] in acetic acid to give the pyrazolo[3,4-*a*]pyrimidines **19**–**21** (Scheme 4), respectively.

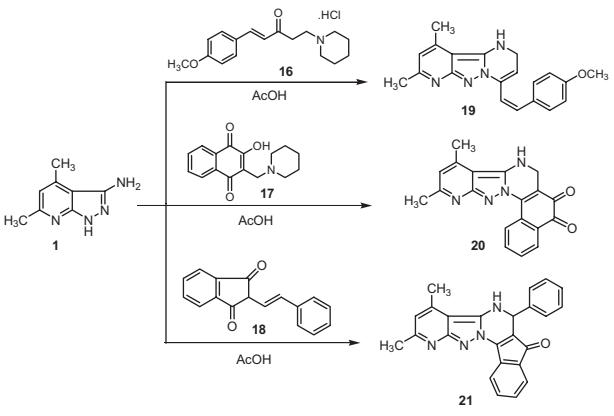
Moreover, treatment of **1** with the enaminone **22** in glacial acetic acid afforded the pyrazolopyrimidine **23**. Furthermore, attempted preparation of the aminopyrazolopyrimidine **25** *via* treatment of **1** with (benzo[*d*]thiazol-2-yl)-3-(dimethylamino)-acrylonitrile (**24**) [31] in glacial acetic acid failed but instead gave the acrylonitrile **26** (Scheme 5).

Furthermore, treatment of **1** with 2-chloroquinoline-3-carbaldehydes **27a** and **b** and 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**28**) [32] in DMF afforded pyrazolopyridine derivatives **29a**,**b** and **30** (Scheme 6), respectively. Cyanoacetylation of **1** with 1-cyanoacetyl-3,5-dimethylpyrazole (**31**) [33] in dioxane afforded the corresponding cyanoacetamide derivative **32** which cyclized to the desired pyrazolopyridine under the influence of glacial acetic acid **33**. Finally, the reactivity of **1** towards *o*-terephthalaldehyde (**34**) was also studied in order to synthesize the diazepine derivative **35**. Thus, *o*-terephthalaldehyde (**34**) was refluxed in glacial acetic acid with **1** to afford **35**.

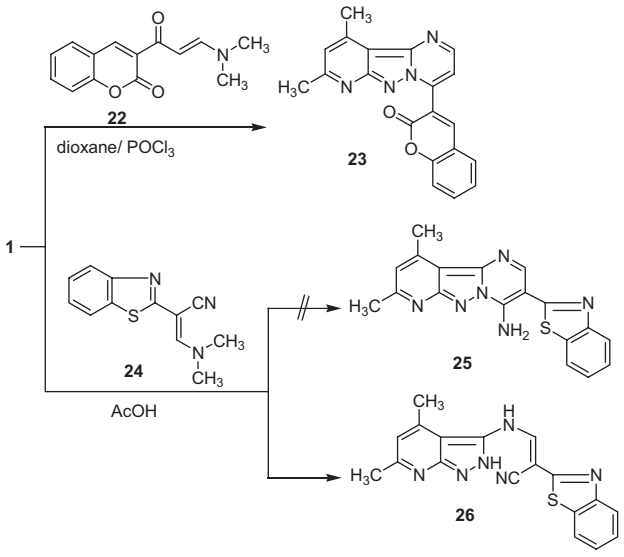
Assignment of the newly synthesized compounds was based on elemental analyses, IR, ^{1}H-NMR and mass spectral data (*c.f.* Experimental part).



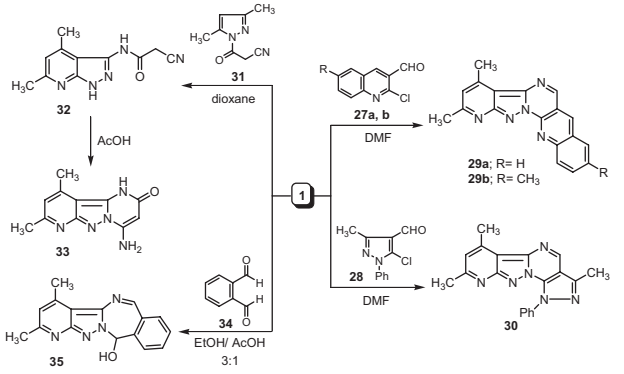
Scheme 2. Reactions of **1** with *p*-quinone derivatives "1,4-diketones".



Scheme 4. Reactions of **1** with Mannich bases and arylidene derivatives.



Scheme 5. Reactions of **1** with *N,N*-dimethyletheneamine derivatives.



Scheme 6. Reactions of **1** with different aldehyde and ketones.

Biological activity

ABTS Antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated by the method of Lissi *et al*. [34]. The antioxidant activity assay employed here is one of the several assays that depend on measuring the consumption of stable free radicals, *i.e*. they evaluate the free radical scavenging activity of the investigated component. The methodology assumes that the consumption of the stable free radical (X′) will be determined by reactions as follows:

$$XH + Y' \rightarrow X' + YH \quad (1)$$

The rate and/or the extent of the process measured in terms of the decrease in X′ concentration would be 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. The assay employs the radical cation derived from 2,2′-azino-bis-(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) as stable free radical to assess the antioxidant potential of the investigated compounds.

Some of the pyrazolopyridine derivatives exhibited an antioxidant effect as shown in Table 1. Compared with the control (ascorbic acid), the antioxidant potency of compounds **1** and **8** was found to be highest, while compounds **10**, **11**, **19** and **35** showed a moderate antioxidant activity and the rest of the compounds tested showed weak antioxidant activity. Compound **1** exhibited a high antioxidant activity compared to the new synthesized compounds.

Bleomycin-dependent DNA damage assay

The compounds pyrazolopyridines were also to test for bleomycin-dependent DNA damage (Table 1) and showed that compounds **1**, **14**, **15**, **23**, **26**, **29a**, **30** and **32** have an ability to protect DNA from damage induced by bleomycin. Also, compound **1** exhibited a high antioxidant activity compared to the new synthesized compounds. By comparing the results obtained for the antioxidant properties of the compounds reported in this study with their structures (Fig. 1), the following structure activity relationships (SARs) were postulated: (i) 3-Aminopyrazolopyridines **1** and **33** are more potent than ascorbic acid which may be attributable to presence of NH and pyrazolopyridine moieties. (ii) Compounds **29a**,**b**, **30** and **32** are more potent or of similar potency as ascorbic acid which may be due to presence of pyrazolopyridopyrimidine moiety. (iii) Compound **23** has good antioxidant activity which may be due to presence of coumarin and pyrazolopyridopyrimidine moieties (Fig. 1).

Conclusion

Some of the tested compounds gave good activity by bleomycin-dependent DNA damage assay than ABTS antioxidant assay because the addition of aqueous buffer solution (pH 7) re-precipitated the compound, so the antioxidant activity decreased compared to that in case of bleomycin-dependent DNA damage.

Experimental

All melting points are recorded on Gallenkamp electric melting point apparatus and are uncorrected. The IR spectra ν (cm^{-1}) (KBr) were recorded on a Perkin Elmer infrared spectrophotometer Model 157. The ^{1}H-NMR spectra of compounds **7**, **12**, **20**, **26** and **29a** were recorded on a Bruker 400 MHz spectrometer and were carried out at the Georgia State University, Atlanta, GA, while the ^{1}H-NMR spectra for the rest of the compounds were obtained on a JEOL spectrophotometer at 500 MHz, using TMS as an internal reference

Table 1. Assay for ABTS antioxidant activity and bleomycin-dependent DNA damage

Compound No.	ABTS		Bleomycin-dependent DNA damage
	Absorbance	%Inhibition	Absorbance
Control	0.498	0.0	–
Ascorbic acid	0.057	88.55	0.098
1	0.065	86.94	0.059
7	0.333	33.31	0.120
8	0.128	74.29	0.134
9	0.342	31.32	0.152
10	0.178	64.25	0.156
11	0.230	53.81	0.122
12	0.426	14.41	0.111
14	0.368	26.10	0.101
15	0.387	22.28	0.100
19	0.298	40.16	0.250
20	0.358	28.11	0.120
21	0.426	14.41	0.134
23	0.324	34.93	0.102
26	0.384	22.89	0.096
29a	0.372	25.30	0.097
29b	0.310	37.75	0.096
30	0.384	22.89	0.099
32	0.320	35.74	0.098
33	0.369	25.90	0.099
35	0.283	43.17	0.160

$$\%\,\text{Inhibition} = (A_{\text{control}} - A_{\text{test}}/A_{\text{control}}) \times 100 \quad \text{Eq. (2)}$$

A_{control}: Absorbance for ascorbic acid
A_{test}: Absorbance for the tested samples
Control: Ascorbic acid

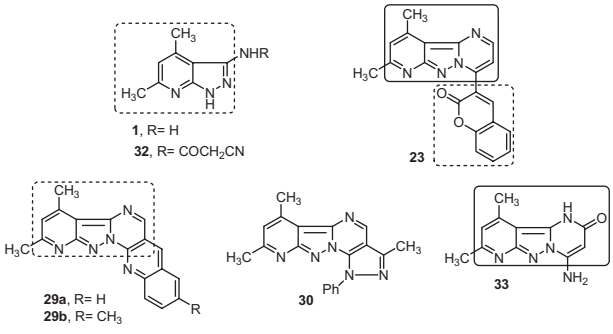


Figure 1. Structure activity relationships of the more potent compounds.

and DMSO-d_6 as solvent, carried out in the National Research Center, Dokki, Giza, Egypt. The mass spectra (EI) were recorded at 70 eV with JEOL JMS600 equipment at the Central Laboratory, Assiut University. Elemental analyses (C, H and N) were carried out at the Microanalytical Center of Cairo University, Giza, Egypt.

*Synthesis of imidazo[1,2-b]pyrazoles **7–11***

To a suspension of **1** (0.32 g, 2 mmol) in DMF/AcOH (11 mL, 10:1), 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**) (0.53 g, 2 mmol), 2-(benzothiazol-2-yl)-4-chloro-3-oxobutanenitrile (**3**) (0.47 g, 2 mmol), 2,3-dibromonaphthalene-1,4-dione (**4**) (0.63 g, 2 mmol), naphtha[2,3-*b*]oxirene-2,7-dione (**5**) (0.63 g, 2 mmol) or 2,5-dichloro-3,6-dihydroxyhexa-2,5-diene-1,4-dione (**6**) (0.42 g, 2 mmol) were added. The reaction mixture was heated under reflux for 6–12 h. The mixture was poured into ice cold-water and neutralized by adding sodium bicarbonate solution. The resulting precipitate was filtered off, dried and crystallized from EtOH/DMF to afford imidazo[1,2-*b*]pyrazole derivatives **7–11**.

*2,4-Dimethyl-6-(2H-chromen-2-on-3-yl)imidazo[1′,2′;2,3]-pyrazolo[5,4-b]pyridine (**7**)*

Reddish brown crystals, reaction time 7 h, yield, 80%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3255 (NH), 1724 (CO), 1615 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.77 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 6.98–8.10 (m, 6H, Ar–H), 8.47 (s, 1H, C-H_4, coumarin), 9.65 (br, s, 1H, NH); MS (EI): *m/z* (%) 330 (M^+, 100%), 313 (3.9), 261 (0.8), 2.28 (0.9), 169 (16.6), 145 (13.3), 100 (13.0), 77 (3.2), 72 (100). Anal. calcd. for $C_{19}H_{14}N_4O_2$ (330.34): C, 69.08; H, 4.27; N, 16.96%. Found: C, 69.12; H, 4.20; N, 16.99%.

*2-(Benzo[d]thiazol-2-yl)2-(2,4-dimethyl-imidazo-[1′,2′;2,3]pyrazolo[5,4-b]pyridin-6-yl-acetonitrile (**8**)*

Brown powder, reaction time 9 h, yield, 77%, mp: >210°C, IR (KBr): ν_{max}, cm^{-1}: 3255 (NH), 2170 (CN), 1620 (C=N); ^{1}H-NMR

(DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 6.54 (s, 1H, CHCN), 7.01–8.03 (m, 6H, Ar–H), 9.59 (br, s, 1H, NH); MS (EI): *m/z* (%) 358 (M^+, 1.3), 323 (0.8), 299 (0.4), 232 (23.3), 212 (14.9), 174 (53.1), 162 (100), 135 (11.6), 107 (11.8), 82 (19.3). Anal. calcd. for $C_{19}H_{14}N_6S$ (358.42): C, 63.67; H, 3.94; N, 23.45%. Found: C, 63.69; H, 3.91; N, 23.5%.

*1-Bromo-2,4-dimethyl-10H-naphthol[1″,2″;4′,5′]-imidazo[1′,2′;2,3]pyrazolo[5,4-b]pyridine-10-one (**9**)*

Reddish brown crystals, reaction time 11 h, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1666 (CO), 1606 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.68 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 7.05–8.11 (m, 5H, Ar–H); MS (EI): *m/z* (%) 381 (M^++2, 0.2), 379 (M^+, 0.2), 282 (100), 239 (0.5), 206 (0.4), 173 (6.1), 108 (18.6), 72 (34.9). Anal. calcd. for $C_{18}H_{11}BrN_4O$ (379.21): C, 57.01; H, 2.92; N, 14.77%. Found: C, 57.08; H, 2.93; N, 14.75%.

*2,4,-Dimethyl-11-hydroxy-10H-naphthol[1″,2″;4′,5′]-imidazo[1′,2′;2,3]pyrazolo[5,4-b]pyridine-10-one (**10**)*

Black powder, reaction time 8 h, yield, 80%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3415 (OH), 1660 (CO), 1613 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.56 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 7.03–8.30 (m, 6H, OH, Ar–H); MS (EI): *m/z* (%) 316 (M^+, 4.0), 314 (22.2), 278 (30.3), 186 (100),162 (47.5), 129 (47.5), 90 (39.4), 79 (65.7), 69 (99.0), 59 (38.4). Anal. calcd. for $C_{18}H_{12}N_4O_2$ (316.31): C, 68.35; H, 3.82; N, 17.71%. Found: C, 68.30; H, 3.69; N, 17.76%.

*7-Chloro-6,9-dihydroxy-2,4-dimethyl-8H-naphthol-[1″,2″;4′,5′]imidazo[1′,2′;2,3] pyrazolo[5,4-b]pyridine-8-one (**11**)*

Black powder, reaction time 13 h, yield, 80%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3239-3199 (br, 2OH), 1640 (CO) and 1618 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 7.03 (s, 1H, Ar–H), 7.13 (br., 1H, OH), 7.23 (br., 1H, OH); MS (EI): *m/z* (%) 318 (M^++2, 0.1), 316 (M^+, 0.2), 282 (100), 251 (0.5), 211 (0.4), 170 (5.8), 147 (3.2), 123 (2.7), 109 (20.4), 75 (7.8), 70 (1.0). Anal. calcd. for $C_{14}H_9ClN_4O_3$ (316.7): C, 53.09; H, 2.86; N, 17.69%. Found: C, 53.05; H, 2.89; N, 17.64%.

*7-Chloro-2,4-dimethyl-8H-naphthol[1″,2″;4′,5′]imidazo-[1′,2′;2,3]pyrazolo[5,4-b] pyridine-8-one-6,9-diyl-diacetate (**12**)*

A suspension of **11** (0.316 g, 1 mmol) in acetic anhydride (10 mL), was refluxed for 3 h. The reaction mixture was poured into ice cold water and then the formed precipitate was filtered off, dried and crystallized from DMF/EtOH to give **12**.

Black powder, yield, 84%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1705 (br, 2CO), 1636 (CO), 1600 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.27 (br, s, 6H, $2COCH_3$), 2.72 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 7.20 (s, 1H, Ar–H); MS (EI): *m/z* (%) 402 (M^++2, 0.6), 400 (M^+, 1.6), 358 (6.8), 282 (100), 268 (0.8), 238 (1.9), 204 (1.6), 170 (10.2), 134 (5.7), 109 (22.2),75 (13.8), 70 (1.3). Anal. calcd. for $C_{18}H_{13}ClN_4O_5$ (400.77): C, 53.94; H, 3.27; N, 13.98%. Found: C, 53.89; H, 3.30; N, 14.02%.

*N-[2-(4,6-Dimethyl-pyrazolo[3,4-b]pyridin-3-yl-imino)-naphthalen-1-oxo-4-yl]-4,6-dimethyl-pyrazolo-[3,4-b]pyridin-3-amine (**14**)*

To a suspension of **1** (1.27 g, 8 mmol) in DMF (15 mL), a solution of 3,4-dihydro-3,4-dioxonaphthalene-1-sulfonate (**13**) (1.04 g, 4 mmol) in H_2O (10 mL) was added. The reaction mixture was heated under reflux for 6 h. The resulting precipitate was filtered off, dried and recrystallized from EtOH/DMF to afford compound **14**.

Red powder, reaction time 6 h, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3434, 3357, 3145 (3NH), 1660 (CO), 1598 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.67 (br, s, 6H, $2CH_3$), 2.82 (br, s, 6H, $2CH_3$), 7.18–7.94 (m, 6H, Ar–H), 8.81 (br, s, 1H, NH), 9.99 (br, s, 1H, NH, pyrazole), 13.04 (br, s, 1H, NH, pyrazole); MS (EI): *m/z* (%) 463 (M^++1, 1.3), 452 (1.7), 387 (100), 358 (75.5), 313 (65.7), 278 (22.6), 262 (18.7), 228 (37.7), 194 (23.4), 161 (40.0), 96 (24.4), 72 (62.3). Anal. calcd. for $C_{26}H_{22}N_8O$ (462.51): C, 67.52; H, 4.79; N, 24.23%. Found: C, 67.50; H, 4.75; N, 24.20%.

*2,4-Dimethyl-10-(4,6-dimethyl-pyrazolo[3,4-b]pyridin-3-yl-imino)-10H-naphthol[1″,2″;4′,5′]imidazo[1′,2′;2,3]-pyrazolo[5,4-b]pyridine (**15**)*

A suspension of **14** (0.463 g, 1 mmol) in a mixture of DMF/AcOH (16 mL, 1:1) was refluxed for 12 h. The resulting precipitate was filtered off, dried and recrystallized from DMF to afford compound **15**.

Reddish brown powder, yield, 79%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1656 (CO), 1600 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (br, s, 6H, $2CH_3$), 2.79 (br, s, 6H, $2CH_3$), 6.94–8.13 (m, 6H, Ar–H), 9.95 (br, s, 1H, NH); MS (EI): *m/z* (%) 444 (M^+, 5.3), 431 (28.9), 400 (5.3), 379 (19.7), 343 (18.4), 298 (17.1), 262 (38.2), 239 (80.3), 186 (56.6), 162 (14.5), 89 (42.1), 72 (100), 56 (55.3). Anal. calcd. for $C_{26}H_{20}N_8$ (444.49): C, 70.26; H, 4.54; N, 25.21%. Found: C, 70.34; H, 4.58; N, 25.25%.

*Synthesis of pyrazolopyrimidine derivatives **19–21** and **26***

To a suspension of **1** (0.528 g, 4 mmol) in glacial acetic acid (25 mL), (*E*)-1-(4-methoxyphenyl)-5-(piperidin-1-yl)-pent-1-en-3-one hydrochloride (**16**) (1.24 g, 4 mmol), 2-hydroxy-3-((piperidin-1-yl)-methyl)-naphthalene-1,4-dione (**17**) (1.08 g, 4 mmol), 2-styryl-2*H*-indene-1,3-dione (**18**) (0.97 g, 4 mmol) or (*E*)-2-(benzol[*d*]thiazol-2-yl)-3-(dimethylamino)-acrylonitrile (**24**) (0.92 g, 4 mmol) were added. The reaction mixture was heated under reflux for 15–20 h (TLC control). The reaction mixture was poured into ice cold-water then the resulting precipitate was filtered off, dried and crystallized from EtOH/DMF to afford compounds **19–21** and **26**, respectively.

*5,6-Dihydro-2,4-dimethyl-8-(4-methoxy-styrenyl-1,5,8a,9-tetrazafluorene (**19**)*

Brown powder, reaction time 15 h, yield, 69%, mp: >310°C, IR (KBr): ν_{max}, cm^{-1}: 3378 (NH), 1615 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 3.70–3.74 (m, 5H, OCH_3, CH_2 of pyrimidine), 6.50–7.41 (m, 9H, NH, 2CH, olefinic, Ar–H); MS (EI): *m/z* (%) 332 (M^++2, 5.7), 330 (M^+, 22.9), 316 (3.8), 279 (8.5), 243 (5.7), 200 (22.5), 161 (100), 120 (43.0), 90 (3.9). Anal. calcd. for $C_{20}H_{20}N_4O$ (332.4): C, 72.27; H, 6.06; N, 16.86%. Found: C, 72.30; H, 6.01; N, 16.91%.

*5,6-Dihydro-2,4-dimethyl-1,5,12a,13-tetraza-indene-[1,2-c]phenanthrene-7,8-dione (**20**)*

Black powder, reaction time 17 h, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3394 (NH), 1668 (br., 2CO), 1616 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.81 (s, 3H, CH_3), 4.76 (br., 2H, CH_2), 6.54 (br., s, 1H, NH), 7.10–8.03 (m, 5H, Ar–H); MS (EI): *m/z* (%) 330 (M^+, 5.7), 328 (8.1), 313 (35.7), 278 (24.6), 253 (15.5), 229 (25.5), 195 (17.2), 179 (23.6), 170 (34.3), 161 (100), 156 (3.7), 96 (31.0), 69 (32.7). Anal. calcd. for $C_{19}H_{14}N_4O_2$ (330.34): C, 69.08; H, 4.27; N, 16.96%. Found: C, 69.06; H, 4.29; N, 17.02%.

*5,6-Dihydro-2,4-dimethyl-6-phenyl-1,5,11a,12-tetraza-indene[1,2-c]fluoren-7-one (**21**)*

Reddish brown powder, reaction time 20 h, yield, 79%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3255 (NH), 1724 (CO), 1615 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.55 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.01 (d, 1H, CH, J = 9.65), 7.03–7.31 (m, 5H, Ar–H), 8.97 (br, s, 1H, NH); MS (EI): *m/z* (%) 330 (M^+, 100%), 313 (3.9), 261 (0.8), 2.28 (0.9), 169 (16.6), 145 (13.3), 100 (13.0), 77 (3.2), 72 (100). Anal. calcd. for $C_{24}H_{18}N_4O$ (378.43): C, 76.17; H, 4.79; N, 14.81%. Found: C, 76.15; H, 4.84; N, 14.86%.

*(E)-3-(4,6-Dimethyl-2H-pyrazolo[3,4-b]pyridin-3-ylamino)-2-(benzo[d]thiazol-2-yl) acrylonitrile (**26**)*

Yellow crystals, reaction time 20 h, yield, 82%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3359, 3237 (2NH), 2206 (CN), 1619 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.10–8.25 (m, 5H, Ar–H), 8.65 (s, 1H, CH), 8.95 (s, 1H, NH), 9.0 (s, NH, pyrazole); MS (EI): *m/z* (%)= 346 (M^+, 17.7), 331 (8.1), 223 (28.8), 179 (10.9), 146 (6.4), 135 (100), 107 (16.0), 88 (2.9). Anal. calcd. for $C_{18}H_{14}N_6S$ (346.41): C, 62.41; H, 4.07; N, 24.26%. Found: C, 62.47; H, 4.15; N, 24.32%.

*Synthesis of 2,4-dimethyl-8-(2H-chromen-2on-3yl)-1,5,8a,9-tetrazafluorene (**23**)*

A mixture of **1** (0.32 g, 2 mmol), **22** (0.46 g, 2 mmol) and phosphorous oxychloride (0.5 mL) in dioxane (20 mL) was refluxed for 6 h on a water bath. The reaction mixture was left to cool and poured gradually onto crushed ice and neutralized with sodium bicarbonate solution to obtain a yellow solid product which was filtered off, washed with water, dried and finally crystallized from DMF/EtOH to give **23**.

Yellow crystals, yield, 77%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1724 (CO), 1606 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.66 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.04–8.56 (m, 8H, Ar–H, C-4 coumarin); MS (EI): *m/z* (%) 342 (M^+, 100%), 315 (32.2), 271 (8.2), 212 (25.3), 162 (20.2), 101 (4.2), 75 (9.3). Anal. calcd. for $C_{20}H_{14}N_4O_2$ (342.35): C, 70.17; H, 4.12; N, 16.37%. Found: C, 70.26; H, 4.17; N, 16.42%.

*Synthesis of pyrazolopyrimidine derivatives **29a,b** and **30***

General procedure

A suspension of compound **1** (0.324 g, 2 mmol and 2-chloroquinoline-3-carbaldehyde (**27a**) (0.382 g, 2 mmol), 6-methyl-2-chloro-quinoline-3-carbaldehyde (**27b**) (0.411 g, 2 mmol) or 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**28**) (0.442 g, 10 mmol) in DMF (15 mL) was heated under reflux with stirring for 7–12 h (TLC control). The reaction mixture was poured onto crushed ice, the formed precipitate filtered off, dried and recrystallized to afford compounds **29a**,**b** and **30**, respectively.

*2,4-Dimethyl-1,5,12,12a,13-pentazaindeno[2,1-a]-anthracene (**29a**)*

Pale yellow powder, reaction time 12 h, crystallization from DMF/EtOH, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1608 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.69 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.12–8.23 (m, 6H, Ar–H), 9.25 (s, 1H, CH=N). MS (EI): *m/z* (%) 299 (M^+, 100), 269 (1.6), 257 (5.5), 230 (9.2), 197 (7.4), 175 (1.7), 140 (1.3), 79 (1.7). Anal. calcd. for $C_{18}H_{13}N_5$ (299.33): C, 72.23; H, 4.38; N, 23.40%. Found: C, 72.17; H, 4.33; N, 23.34%.

*2,4,9-Trimethyl-1,5,12,12a,13-pentazaindeno[2,1-a]-anthracene (**29b**)*

Yellow powder, reaction time 10 h, crystallization from DMF/EtOH, yield, 88%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 1612 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.05 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 7.01–8.26 (m, 5H, Ar–H), 9.43 (br., s, 1H, CH=N); MS (EI): *m/z* (%) 314 (M^+, 100), 289 (1.6), 247 (0.9), 230 (9.2), 197 (62.3), 162 (33.3), 97 (3.1), 72 (8.3). Anal. calcd. for $C_{19}H_{15}N_5$ (313.36): C, 72.83; H, 4.82; N, 22.35%. Found: C, 72.89; H, 4.87; N, 22.46%.

*1,10a-Dihydro-3,6,8-trimethyl-1-phenylcyclopenta[a]-fluorine-3,6,8-trimethyl-1-phenyl-1,2,5,9,10,10a-hexazacyclopenta[a]fluorine (**30**)*

Pale yellow needle crystals, reaction time 7 h, crystallization from benzene/EtOH, yield, 91%, mp: 271°C, IR (KBr):

ν_{max}, cm^{-1}: 1625 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.60 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 7.04–8.19 (m, 6H, Ar–H), 8.53 (s, 1H, CH=N); MS (EI): *m/z* (%) 328 (M^+, 1.6), 218 (21.5), 183 (21.5), 183 (7.5), 141 (6.9), 124 (24.7), 76 (3.1), 72 (100). Anal. calcd. for $C_{19}H_{16}N_6$ (328.37): C, 69.50; H, 4.91; N, 25.59%. Found: C, 69.58; H, 4.88; N, 25.57%.

*Synthesis of 2-cyano-N-(4,6-dimethyl-1H-pyrazolo[3,4-b]-pyridin-3-yl)acetamide (**32**)*

A mixture of **1** (1.62 g, 10 mmol) and 1-cyanoacetyl-3,4-dimethylpyrazole (**31**) (1.63 g, 10 mmol) in dioxane (30 mL) was refluxed for 5 h. The separated precipitate was filtered off, dried and recrystallized from DMF/ethanol to give compound **32**.

White crystals, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3235 (NH); 2258 (CN); 1662 (C=O), 1612 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.63 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 4.23 (s, 2H, CH_2), 7.13 (s, 1H, Ar–H), 10.21 (s, 1H, NH), 13.22 (s, 1H, NHCO); MS (EI): *m/z* (%)= 229 (M^+, 0.5), 228 (M^+–H, 100), 188 (15.2), 161 (92.3), 131 (10.4), 95 (14.6), 76 (5.2). Anal. calcd. for $C_{11}H_{11}N_5O$ (229.24): C, 57.63; H, 4.84; N, 30.55%. Found: C, 57.65; H, 4.50; N, 30.53%.

*Synthesis of 5,6-dihydro-2,4-dimethyl-6-oxo-1,5,8a,9-tetrazafluorene-8-amine (**33**)*

Compound **32** (0.6 g, 2 mmol) in glacial acetic acid (10 mL) was heated under reflux for 12 h. The reaction mixture was cooled and the solid product collected by filtration and crystallized from DMF to give compound **33**.

Pale yellow powder, yield, 90%, mp: >320°C, IR (KBr): ν_{max}, cm^{-1}: 3399, 3376, 3315 (NH_2, NH), 1664 (C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.95 (br, s, 2H, NH_2), 6.65 (s, 1H, CH, pyrimidine ring), 6.49 (br, s, 1H, NH), 7.07 (s, 1H, Ar–H). Anal. calcd. for $C_{11}H_{11}N_5O$ (229.24): C, 57.63; H, 4.84; N, 30.55%. Found: C, 57.60; H, 4.90; N, 30.57%.

*Synthesis of 11,11a-dihydro-2,4-dimethyl-1,5,11a,12-tetraza-benzo[c]benzo[f]azulen-11-ol (**35**)*

A mixture of **1** (0.324 g, 2 mmol) and phthaladehyde (**34**) (0.268 g, 2 mmol) was refluxed in a mixture of ethanol/acetic acid (20 mL, 3:1) for 5 h. The product mixture was poured gradually onto crushed ice with vigorous stirring to obtain a yellow solid product, filtered off, washed with water, dried and finally crystallized from DMF/EtOH to give compound **35**.

Yellow crystals, yield, 89%, mp: >300°C, IR (KBr): ν_{max}, cm^{-1}: 3376 (OH), 1606 (C=N); ^{1}H-NMR (DMSO-d_6): δ 2.70 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 6.93–8.09 (m, 7H, Ar–H, CH=N, OH); MS (EI): *m/z* (%) 278 (M^+, 5.6), 261 (M^+–OH, 1.7), 230 (2.5), 161 (4.6), 147 (28.1), 95 (100), 75 (65.3). Anal. calcd. for $C_{16}H_{14}N_4O$ (278.31): C, 69.05; H, 5.07; N, 20.13%. Found: C, 69.09; H, 5.10; N, 20.16%.

Materials and methods

Antioxidant screening

Antioxidant activity screening assay; ABTS method [34]

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation derived from ABTS was prepared by reaction of ABTS (60 μl) with MnO_2 (3 mL, 25 mg/mL) in (5 mL) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered. The absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was recorded at λ_{max} 734 nm. The absorbance (A_{test}) was measured upon the addition of (20 μL of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The inhibition ratio (%) was calculated using the following formula:

$$\%\,\text{Inhibition} = (A_{control} - A_{test}/A_{control}) \times 100 \quad (2)$$

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

Bleomycin-dependent DNA damage

The assay was performed according to Aeschlach *et al.* [35] and Chan & Tang [36], with minor modifications. L-Ascorbic acid was used as a positive control. The tested compounds were dissolved in DMSO (1 mg/mL). A mixture of DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and the sample (20 μL) was prepared. The previous mixture (0.5 mL) was incubated at 37°C for 1 h, then 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 absorbance of the tested compounds was measured at λ_{max} 532 nm the extent of DNA damage was measured by the increase in absorbance.

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References

[1] S. O. Elham, *Acta Chim. Slov.* **2003**, *50*, 15–28.

[2] J. J. Baldwin, P. K. Lumma, F. C. Novello, G. S. Ponticello, J. M. Sprague, D. E. Duggan, *J. Med. Chem.* **1977**, *20*, 1189–1193.

[3] J. R. Beck, M. P. Lynch, G.B. Patent, **1985**, 2,149,402; [*Chem. Abstr.* **1985**, *103*, 141938].

[4] I. Okada, S. O. Kui, Y. Takahashi, T. Fukuchi, E.P. Patent, **1989**, 289,879; [*Chem. Abstr.* **1989**, *110*, 96234].

[5] A. Gürsoy, S. Demirayak, G. Çapan, K. Erol, K. Vural, *Eur. J. Med. Chem.* **2000**, *35*, 359–364.

[6] Ş. G. Küçükgüzel, S. Rollas, H. Erdeniz, M. Kiraz, A. C. Ekinci, A. Vidin, *Eur. J. Med. Chem.* **2000**, *35*, 761–771.

[7] T. M. Bare, C. D. McLaren, J. B. Campbell, J. W. Firor, J. F. Resch, C. P. Walters, A. I. Salama, B. A. Meiners, J. B. Patel, *J. Med. Chem.* **1989**, *32*, 2561–2573.

[8] A. M. R. Bernardino, A. R. Azevedo, L. C. S. Pinheiro, J. C. Borges, V. L. Carvalho, M. D. Miranda, M. D. F. Meneses, M. Nascimento, D. Ferreira, M. A. Rebello, V. A. G. G. Silva, I. C. P. P. Frugulhetti, *Med. Chem. Res.* **2007**, *16*, 352–369.

[9] A. M. R. Bernardino, H. C. Castro, I. C. P. P. Frugulhetti, N. I. V. Loureiro, A. R. Azevedo, L. C. S. Pinheiro, T. M. L. Souza, V. Giongo, F. Passamani, U. O. Magalhães, M. G. Albuquerque, L. M. Cabral, C. R. Rodrigues, *Bioorg. Med. Chem.* **2008**, *16*, 313–321.

[10] H. de Mello, A. Echevarria, A. M. Bernardino, M. Canto-Cavalheiro, L. L. Leon, *J. Med. Chem.* **2004**, *47*, 5427–5432.

[11] Z. Lu, G. R. Ott, R. Anand, R.-Q. Liu, M. B. Covington, K. Vaddi, M. Qian, R. C. Newton, D. D. Christ, J. Trzaskos, J. J.-W. Duan, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1958–1962.

[12] M. A. Gouda, A. A. Abu-Hashem, *Arch. Pharm.* **2011**, *344* (*3*), 170–177.

[13] S. A. A. El Bialy, M. A. Gouda, *J. Heterocycl. Chem.* **2010**, JHET634, DOI: 10.1002/jhet.634.

[14] W. S. Hamama, M. A. Berghot, E. A. Baz, M. A. Gouda, *Arch. Pharm.* **2011**, DOI: 10.1002/ardp.201000263.

[15] M. A. Gouda, M. A. Berghot, E. A. Baz, W. S. Hamama, *Med. Chem. Res.* **2011**, published online. DOI: 10.1007/s00044-011-9610-8.

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

[17] N. Siddiqui, M. F. Arshad, S. A. Khan, *Acta Poloniae Pharm. Drug Res.* **2009**, *66*, 161–167.

[18] K. S. Mohamed, *Ph. D. Thesis*, Mansoura University, Mansouri, Egypt **2001**.

[19] S. M. McElvain, E. L. Engelhardt, *J. Am. Chem. Soc.* **1944**, *66*, 1077–1083.

[20] R. L. Betts, S. T. Murphy, C. R. Johnson, *Tetrahedron: Asymm.* **2004**, *15*, 2853–2860.

[21] M. Khalil, M. A. Berghot, M. A. Gouda, *Eur. J. Med. Chem.* **2010**, *45*, 1552–1559.

[22] A. Bendich, P. J. Russell, Jr, J. J. Fox, *J. Am. Chem. Soc.* **1954**, *76*, 6073–6077.

[23] S. Kobayashi, *Chem. Pharm. Bull.* **1973**, *21*, 941–951.

[24] R. A. Long, J. F. Gerster, L. B. Townsend, *J. Heterocycl. Chem.* **1970**, *7*, 863–869.

[25] T. Novinson, R. Hauson, M. K. Dimitt, L. N. Simon, R. K. Robins, D. E. O'Brien, *J. Med. Chem.* **1974**, *17*, 645–648.

[26] G. Tacconi, G. Gatti, G. Desimoni, V. Messori, *J. Prakt. Chem.* **1980**, *322*, 831–834.

[27] M. B. El-Kashef, M. A. Abdalla, B. E. Bayoumi, A. A. M. El-Timawy, *J. Chem. Technol. Biotechnol.* **1983**, *33*, 294–298.

[28] M. L. Edwards, H. W. Ritter, D. M. Stemerick, K. T. Stewart, *J. Med. Chem.* **1983**, *26*, 431–436.

[29] H. Dam, J. Glavind, P. Karrer, *Helv. Chim. Acta* **1940**, *23*, 224–233; [*Chem. Abstr.* **1940**, *34*, 5493].

[30] L. Geita, G. Vanags, *Zhur. Obshchei Khim.* **1957**, *27*, 3109–14; [*Chem. Abstr.* **1958**, *52*, 9111].

[31] K. M. Dawood, Z. E. Kandeel, A. M. Farag, *J. Chem. Res. Synop.* **1998**, (*4*), 208–209; [*Chem. Abstr.* **1998**, *129*, 67759e].

[32] R. A. Pawer, A. A. Patil, *Indian J. Chem.* **1994**, *32B*, 156–158.

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

[34] E. A. Lissi, B. Modak, R. Torres, J. Esocbar, A. Urzua, *Free Radical Res.* **1999**, *30*, 471–477.

[35] R. Aeschbach, J. Löliger, B. C. Scott, A. Murcia, J. Butler, B. Halliwell, O. I. Aruoma, *Food Chem. Toxicol.* **1994**, *32*, 31–36.

[36] T. Y. Chan, P. L. Tang, *J. Pineal Res.* **1996**, *20*, 187–191.

