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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SOME PHENOLIC COMPOUNDS INCORPORATING PYRAZOLE MOIETY

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ABSTRACT

4-Arylidene-3-methyl-1-phenyl-5-pyrazolones **2a-c** were treated with phenols such as resorcinol, phloroglucinol, pyrogallol, 8-hydroxyquinoline, orcinol, 2-naphthol and 1,5-dihydroxynaphthalene to give a series of phenolic compounds incorporating pyrazole moiety or 1,4-dihydrochromeno[2,3-*c*]pyrazole derivatives, depending on the reaction conditions. In addition, compounds **2a** and **2c** were subjected to Mannich reaction to give the phenolic bases **20**-**22** and bis-bases **24a** and **24b**. The newly synthesized compounds were screened for their antioxidant activity and Bleomycine-dependent DNA damage assay.

Key words: phenolic compounds, pyrazoles, antioxidant activity

INTRODUCTION

A variety of compounds having a 5-pyrazolone moiety as a structural unit have been synthesized and studied with interest centered on their potential pharmaceutical activity. The literature survey reveals that the pyrazolone moiety is an important pharmacophore **(Mariappan, et al., 2010)** and exhibits outstanding biological activities such as, antibacterial, antifungal **(Al-Haiza, et al., 2001)**, anti-inflammatory **(Mariappan, et. al., 2010 and Badawey, et al., 1998)**, analgesic **(Mohd & Kumar, 2005; and Gursoy, et al., 2000), antitubercular (Joshi et**

al., 2007, Castagnolo, et al., 2009), antioxidant **(Kumar, et al., 2008)** and anticancer **(Sunil, et al., 2009)** activities.

On the other hand, phenolic compounds are important pharmacophores in the medicinal and pharmaceutical fields. Phenolic compounds (e.g. phenolic acids, flavonoids, coumarins and tannins) possess a potential antioxidant activity **(Larson**, **1988; Cotelle, et al., 1996; Velioglu, et al., 1998; Zheng & Wang 2001 and Cai, et al., 2004)**, and many of these compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial or antiviral activities **(Owen, et al., 2000 and Sala, et al., 2002).**

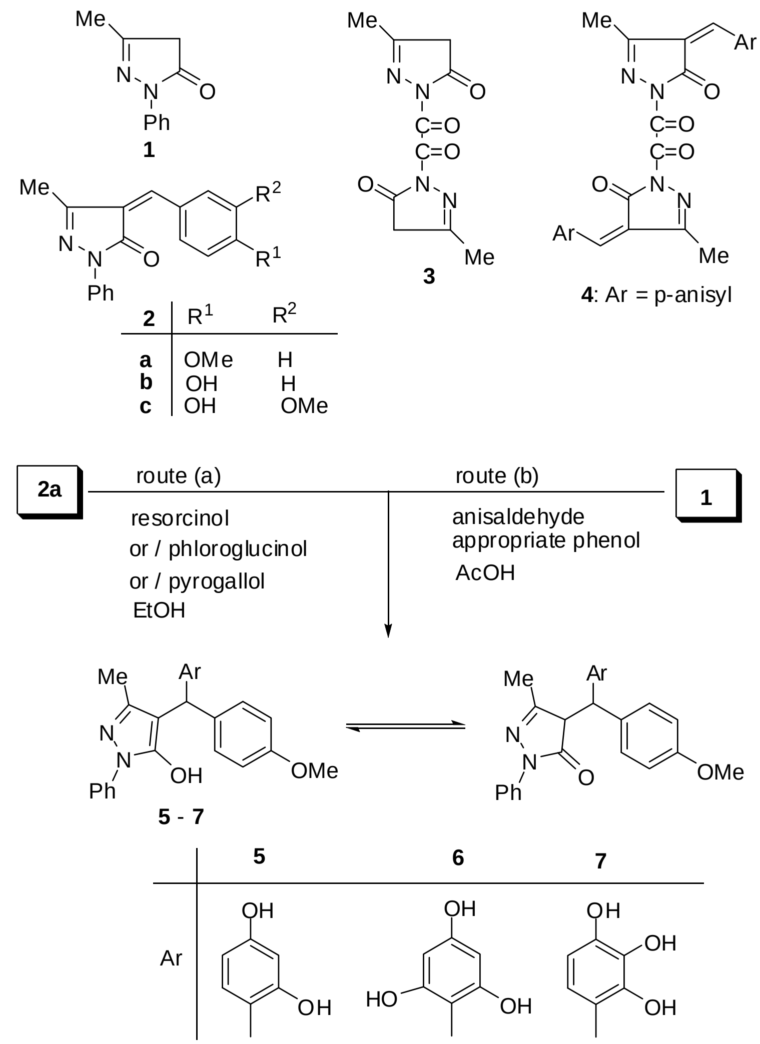
From the view point of molecular design, the combination of two biologically active molecules or pharmacophores is a well-known approach for the build-up of drug-like molecules, which allows us to find more potent agents. The present study deals with the synthesis of some phenolic compounds incorporating pyrazole moiety, starting from 4-arylidene-3-methyl-1-phenyl-5-pyrazolones **2a-c** as key starting compounds. The new products might possess considerable synthetic and pharmaceutical interest.

RESULTS AND DISCUSSION

In the present study, 4-arylidene-3-methyl-1-phenyl-5-pyrazolones **2a-c** were prepared by treating 3-methyl-1-phenyl-5-pyrazolone (**1**) with the appropriate aldehyde as previously described **(Sawedy, et al., 1950; Afsah, et al., 1980 and Amal & Kapuano 1951)**. The reaction of oxalyl-1,1'-bis(3-methyl-2-pyrazolin-5-one) (**3**) **(Ram & Pandey, 1975)** with *p*-anisaldehyde gave the bis[4-(4-methoxybenzylidene)] derivative **4**, which is insoluble in most organic solvents.

Treatment of **2a** with dihydric or trihydric phenols such as resorcinol, phloroglucinol and pyrogallol afforded the corresponding phenolic compounds **5**, **6** and **7**, respectively. In addition, the alternative synthesis of compounds **5-7** by one-pot, four-component sequential reaction of 5-pyrazolone (**1**), *p*-anisaldehyde and the appropriate phenol, confirmed their structures (route b). The formation of **5-7** *via* route (b), is in line with a recent report **(Gunasekaran, et al., 2011)** on the reaction of **1** with aromatic aldehydes and β-naphthol to give 4-[(2-hydroxy-1-naphthyl)arylmethyl]pyrazoles.

The intermediacy of the 4-(4-methoxybenzylidene) derivative **2a** in this sequential reaction is evident from the formation of **5-7** *via* route (a). According to previous studies **(Gunasekaran, et al., 2011; Elguero, et al., 1976; Hwang, et al., 1993; Alderete, et al., 2000;Belmar;et al., 2001 and Belmar;et al., 1999)** pyrazolones could exist in three tautomeric forms, *viz*. the CH, OH and NH forms **(Fig. 1).** The tautomers between the OH and CH forms have been reported to exist in solution and even in crystals **(Elguero, et al., 1976).**



Scheme 1

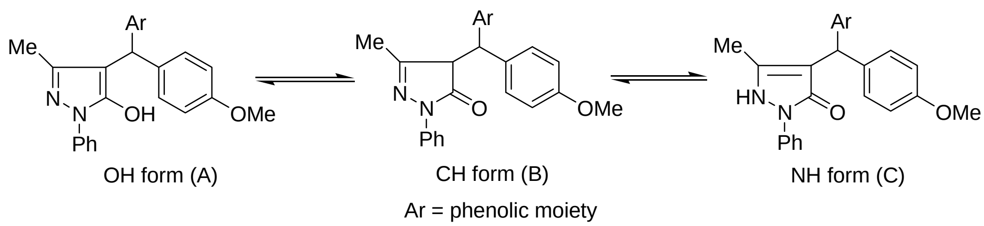
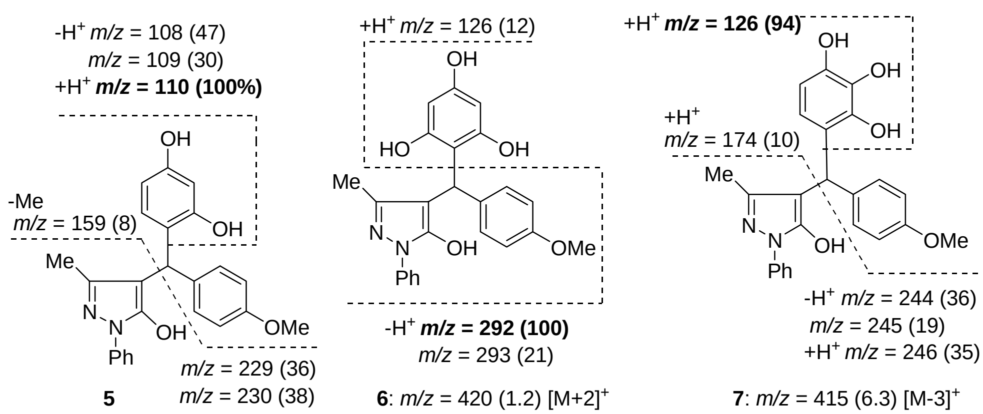


Fig. 1. Tautomeric forms of pyrazolones (**5**-**7**)

The IR spectral data of compounds **5**, **6** and **7** showed absorption bands at 1602- 1606 (C=N) and 3400-3445 cm^{-1} due to (OH), and no absorption attributed to (C=O) group, indicating that compounds **5-7** exist as a 5-hydroxypyrazole form (A). The main characteristic features of the ^{1}H NMR spectrum of **7** as an example, are a singlet at δ = 5.94 assignable to (CH), three singlets at 5.72, 5.59 and 5.19 (3 x OH), a broad singlet at 7.54 (enolic OH), two singlets at 3.69 (OCH_3) and 2.48 (CH_3). The mass spectra of **5-7** showed very similar cleavage patterns. Cleavage at the branched carbon atom with elimination of phenolic ion leads to the base peak at *m/z* = 110 due to resorcinol ion (for **5**), 292 [M-phloroglucinol ion] (for **6**) and a very intense peak at *m/z* = 126 (94%) due to pyrogallol ion (for **7**), as depicted in Scheme 2.

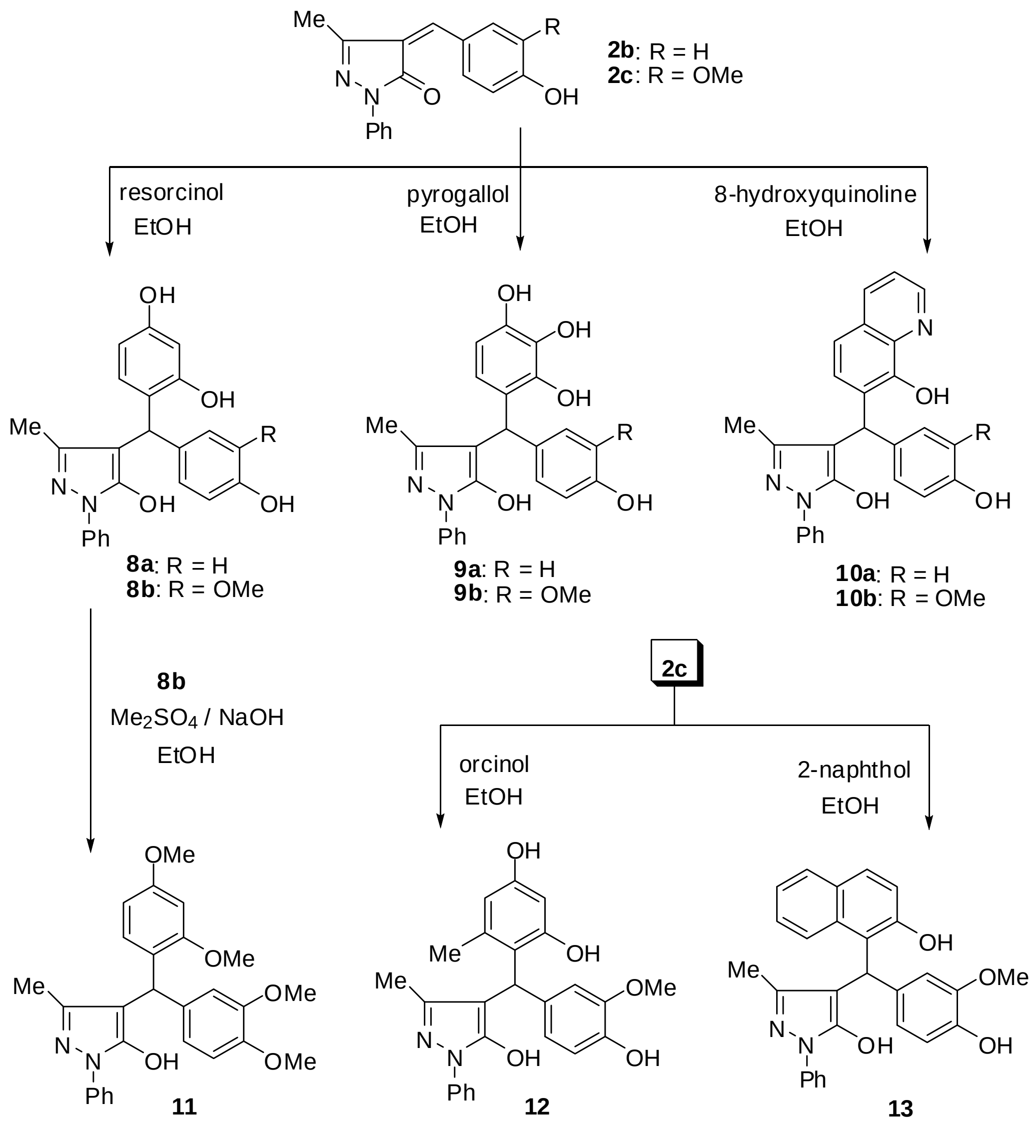


Scheme 2

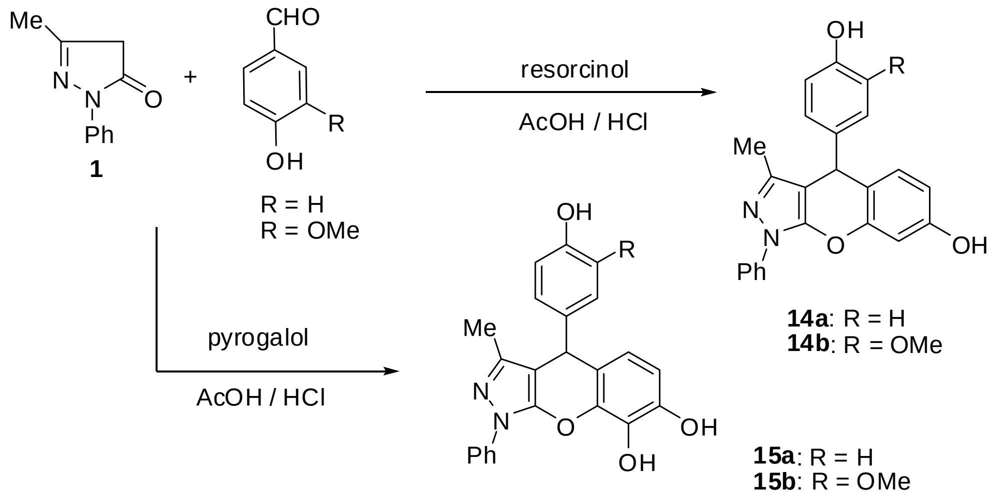
This reaction with phenols has been extended to 4-(4-hydroxybenzylidene)-3-methyl-1-phenyl-5-pyrazolone (**2b**) and the 4-(4-hydroxy-3-methoxybenzylidene) analog (**2c**), thus enabling the formation of di-phenolic compounds through a Michael type reaction. Therefore, the synthesis of 4-[(2,4-dihydroxyphenyl)(4-hydroxyphenyl)methyl]-3-methyl-1-phenyl-1*H*-pyrazol-5-ol (**8a**) and the 4-(4-hydroxy-3-methoxyphenyl) analog (**8b**) has been achieved by the reaction of resorcinol with **2b and 2c**, respectively. A similar reaction takes place on treating **2b** and **2c** with pyrogallol and 8-hydroxyquinoline, yielding compounds **9a**-**b** and **10a**-**b**. In addition, treatment of **8b** with dimethyl sulfate afforded 4-[(2,4-dimethoxyphenyl)(3,4-dimethoxyphenyl)methyl]-3-methyl-1-phenyl-1*H*--pyrazol-5-ol (**11**) (Scheme 3).

In line with this, 4-[(2,4-dihydroxy-6-methylphenyl)(4-hydroxy-3-methoxyphenyl)methyl]-3-methyl-1-phenyl-1*H*-pyrazol-5-ol (**12**) and the (2-hydroxynaphthalen-1-yl)methyl analog (**13**) were obtained by treating **2c** with orcinol and 2-naphthol, respectively. The mass, IR and ^{1}H NMR spectral data of compounds **8-13** are consistent with their structures.

On the other hand, the one-pot, three-component sequential reaction of 5-pyrazolone (**1**), *p*-hydroxybenzaldehyde and/or vanillin with resorcinol in acidic medium proceeded smoothly to give 4-(4-hydroxyphenyl)-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-*c*]pyrazol-7-ol (**14a**) and the 4-(4-hydroxy-3-methoxyphenyl) analog (**14b**), respectively. A similar reaction takes place on using pyrogallol, yielding 4-(4-hydroxyphenyl)-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-*c*]pyrazole-7,8-diol (**15**a) and the 4-(4-hydroxy-3-methoxyphenyl) analog (**15b**) (Scheme 4). The formation of compounds **14a**,**b** and **15a**,**b** is in line with the reported synthesis of benzochromeno-pyrazoles *via* reaction of aldehydes with **1** and α- or β-naphthol **(Heravi, et al., 2011)**. The ^{1}H NMR spectrum of **15b**, as an example, revealed a singlet at δ = 6.07 assignable to (4-H), three singlets at 6.04, 5.56 and 5.54 (3 x OH), two singlets at 3.42 (OCH_3) and 2.49 (CH_3). The mass spectra of **14a**,**b** and **15a**,**b** contain peaks of the respective molecular ions, and fragmentation patterns which supported their structures.


Me
R
N
N
O
Ph
OH
2b: R = H
2c: R = OMe
resorcinol
EtOH
pyrogallol
EtOH
8-hydroxyquinoline
EtOH
8a: R = H
8b: R = OMe
9a: R = H
9b: R = OMe
10a: R = H
10b: R = OMe
8b
Me2SO4 / NaOH
EtOH
2c
orcinol
EtOH
2-naphthol
EtOH
OMe
11
12
13

Scheme 3

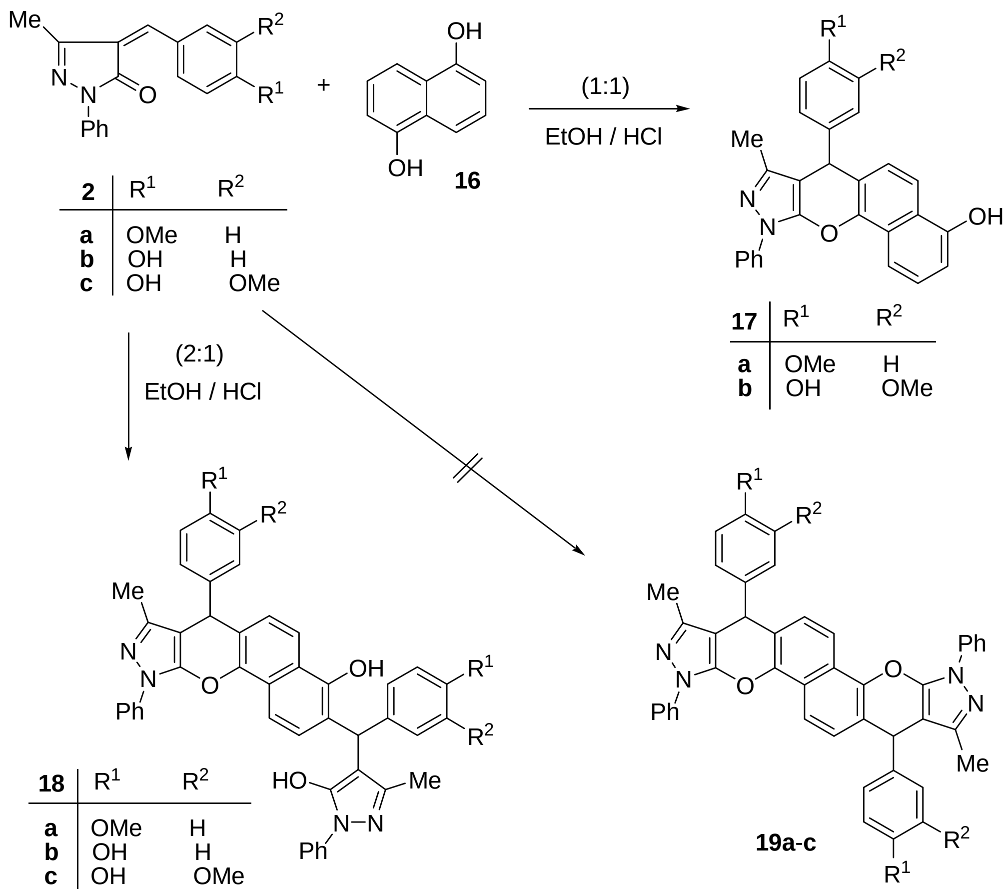


Scheme 4

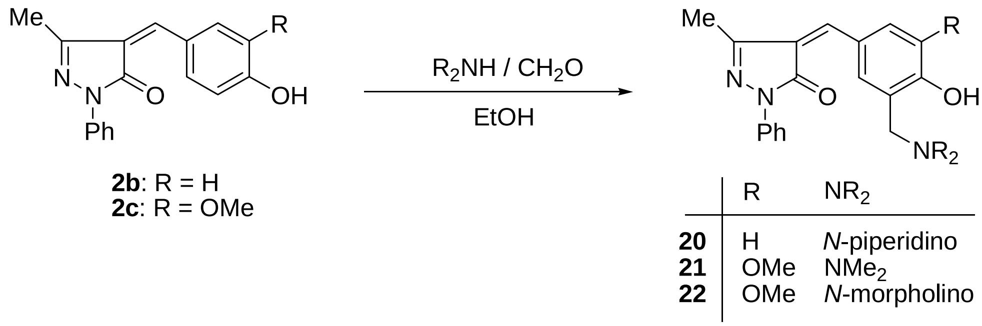
The scope of the above reaction has been broadened by treatment of **2a** and **2c** with 1,5-dihydroxynaphthalene (**16**) in acidic medium to give 7*H*-(7-*p*-anisyl)-8-methyl-10-phenyl-pyrazolo[5,4-*b*]benzo[h] chromen-4-ol (**17a**) and the 7-(4-hydroxyl-3-methoxyphenyl) analog (**17b**), respectively. Whereas, the reaction of **2a-c** with **16** in a molar ratio of 2:1 afforded **18a-c**, respectively, rather than the expected polycyclic compounds **19a-c**, as confirmed by analytical and spectral data.

A variety of compounds having a phenolic Mannich base as a structural unit have been synthesized and studied with interest centered on their potential pharmaceutical activity. In particular, a number of phenolic Mannich bases and bis(Mannich bases) related to chalcones have demonstrated significant cytotoxicity and anticancer properties **(Gul, et al., 2008; Reddy, et al., 2008 and Saydam, et al., 2003).**

In view of this, the Mannich reaction of the phenolic moiety of compounds **2b** and **2c** is of particular interest, because it provides access to 4-arylidene-3-methyl-1-phenyl-5-pyrazolones possessing a phenolic Mannich base as a structural unit. This has been realized by treating **2b** with piperidine and formaldehyde to give 4-[4-hydroxy-3-(piperidin-1-ylmethyl)benzylidene]-3-methyl-1-phenyl-5-pyrazolone (**20**). The analogous reaction of **2c** with dimethylamine and morpholine gave the phenolic Mannich bases **21** and **22**, respectively (Scheme 6).

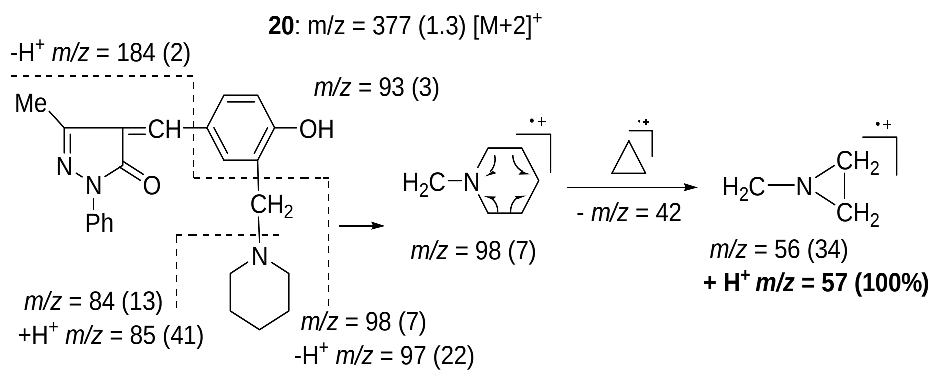

Me
R2
R1
N
N
O
Ph
+
OH
OH
16
(1:1)
EtOH / HCl
2 | R1 | R2
a | OMe | H
b | OH | H
c | OH | OMe
17 | R1 | R2
a | OMe | H
b | OH | OMe
(2:1)
EtOH / HCl
18 | R1 | R2
a | OMe | H
b | OH | H
c | OH | OMe
HO
Me
OH
19a-c

Scheme 5



Scheme 6

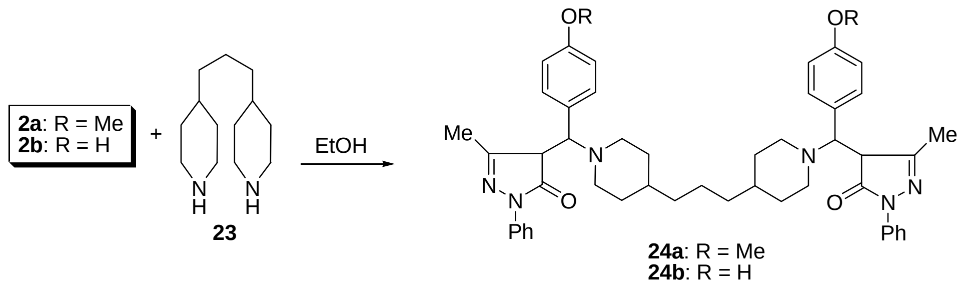
The mass spectra of compounds **20**, **21** and **22** contain peaks of the respective molecular ions, and fragmentation patterns which supported their structures. The mass spectrum of **20**, as an example, revealed a molecular ion peak at m/z = 377 $[M+2]^+$, the basic side chain can be identified by two peaks at m/z = 85 (41%) and 97 (22%) due to *N*-piperidinomethyl ion, which undergo further fragmentation to give the base peak at m/z = 57 (100%), as depicted in Scheme 7.



Scheme 7

In connection with this study, the reaction of compounds **2a** and **2b** with 4,4'-trimethylenedipiperidine (**23**) was investigated as a route to bis-(Mannich bases) of the type **24** having two pyrazolone units. This has been achieved by treating **2a** and **2b** with **23** to give N,N'-di(4-methoxyphenyl-3-methyl-1-phenyl-5-pyrazolone-4-ylmethyl)-4,4"-trimethylenedipiperidine (**24a**) and the N,N'-di(4-hydroxyphenyl-3-methyl-1-phenyl-5-pyrazolone-4-ylmethyl) analog (**24b**), respectively

(Scheme 8). The analytical, IR, ^{1}H and ^{13}C NMR and mass spectral data are consistent with the structures proposed for compounds **24a** and **24b**.



Scheme 8

Biological Activity

Table (1): ABTS Antioxidant activity assay

Compound No.	Absorbance of Sample	Compound No. % inhibition
L-Ascorbic acid	1.04	80
4	0.24	35.4
6	2.01	61.10
7	0.18	75.4
8a	0.05	37.5
8b	0.1	34.3
13	0.05	37.5
14a	0. 7	54.2
14b	0.7	44.2
15a	0.7	54.2
15b	0.1	84.3
17a	0.01	38.3
17 b	0.13	36.8
18a	0.14	34.2
18b	0.13	56.8
18c	2.38	41.1
20	1.30	45.8
21	1.30	45.8
24a	1.11	25.8

ABTS Antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated by the ABTS method **(El-Gazzar, et al., 2009).** The obtained data (Table 1) showed that:

a. Compounds **7** and **15b** proved to exhibit the highest activity
b. Compounds **14a**, **15a** and **18b** showed a moderate antioxidant activity.
c. The rest of the tested compounds revealed weak activity.

The presence of 5-pyrazolone moiety implies its importance in the antioxidant effect.

The structure activity relationship (SAR) of the tested compounds indicate that the presence of methoxy group increased the antioxidant activity. The antioxidant activity increased with the introduction of one methoxy group and three hydroxyl groups as shown in **7** in addition to the presence of chromene group in **15b.**

Compound **7** possessed the best substitution on the pyrazole ring causing high antioxidant activity which was the 2,3,4-trihydroxyphenyl group.

For compound **15b** having the dihydrochromene pyrazole moiety the best substitution causing high antioxidant activity was 4-hydroxy-3-methoxy groups at the phenolic moiety

Compounds **4, 6, 7, 8a, 8b, 13, 14a, 14b, 15a, 15b, 17a, 17b, 18a, 18b, 18c, 20, 21** and **24a** were selected to test for Bleomycin-dependent DNA damage (Table 2). Damage to DNA in the presence of Bleomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents **(Gutteridge, et al., 1981)**. If the samples to be tested are able to reduce the Bleomycin-Fe^{3+} to Bleomycin-Fe^{2+}, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. L-Ascorbic acid can reduce Fe^{3+} to Fe^{2+} as a reducing agent.

Table 2 shows that compounds **4, 7, 8a, 13, 14a, 14b, 15a, 17a, 17b, 18b, 20** and **21** have an ability to protect DNA from the induced damage by bleomycin. From the structure activity relationship (SAR), it is noteworthy that compounds **4, 7, 8a, 13, 14a, 14b, 15a, 17a, 17b, 18b, 20** and **21** have OMe, OH, $(Me)_2NH$ and piperidine groups attached to aromatic ring which is effective in protecting DNA damage from Bleomycin.

Table (2): Bleomycin-dependent DNA damage activity

Compound No.	Bleomycine-dependent DNA damage Absorbance of samples
L-Ascorbic acid	0.0038 ± 0.01
4	0.017 ± 0.05
6	0.080 ± 0.22
7	0.015 ± 0.04
8a	0.017 ± 0.05
8b	0.210 ± 1.12
13	0.017 ± 0.05
14a	0.016 ± 0.27
14b	0.016 ± 0.27
15a	0.016 ± 0.27
15b	0.210 ± 1.12
17a	0.016 ± 2.04
17 b	0.017 ± 0.04
18a	0.230 ± 1.14
18b	0.017 ± 0.04
18c	0.080 ± 0.20
20	0.014 ± 1.31
21	0.014 ± 1.31
24a	0.116 ± 1.27

Table (3): Lymphocyte transformation

Compound No.	Lymphocyte transformation assay, at 50 µM
Echinacea purpurea	74
4	20
6	45
7	59
8a	30
8b	10
13	30
14a	45
14b	45
15a	45
15b	65
17a	15
17 b	35
18a	10
18b	35
18c	25
20	65
21	65
24a	35

Lymphocyte transformation assay

The lymphocyte transformation (mitogensis) or proliferation assay was used to assess the immnunomodulating activity of compounds **4, 7, 8a, 13, 14a, 14b, 15a, 17a, 17b, 18b, 20** and **21**. The cell-mediated immune response was determined in the peripheral blood lymphocytes (PBL) in response to mitogenic stimulation using either phytohaemagglutinin (PHA) or concanavalin A (Con A) as mitogens that stimulate human T and B cells but T-cells more vigorously. Table 3 shows that compounds **7, 15b, 20** and **21** have the highest ability for transformation (proliferated) blasts.

CONCLUSION

The newly prepared compounds showed interesting biological activities. Compound **7** exhibited a high antioxidant activity and best protective effect against DNA damage induced by bleomycin.

Experimental Section

All melting points (uncorrected) were determined on a Gallenkamp electric melting point apparatus. Elemental microanalyses were carried out at Microanalytical Unit, Mansoura University. Infrared spectra were measured on a Mattson 5000 FTIR spectrometer. ^1H and ^{13}C NMR data were obtained in $CDCl_3$ or $[D_6]$ DMSO solution on a Varian XL 200 MHz instrument using TMS as internal standard. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Mass spectra were recorded on a GC-MS QP –1000 EX Shimadzu instrument. The course of the reaction and the purity of the synthesized compounds were monitored by TLC using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp.

1,2-Bis[4-(4-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl]ethane-1,2-dione (4)

A mixture of **3** (2.5 g, 0.01 mol) and *p*-anisaldehyde (2.36 g, 0.02 mol) was heated at 125 ^oC (oil bath) for 40 min. The product that was obtained on cooling was washed with boiling ethanol to give **4**. M. p. 320^{o}C. Yield 75 % (white powder). − IR (KBr): ν = 1660, 1607, 1500, 1442, 1319, 1242, 1118 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 487 (23) $[M+1]^+$, 437 (19), 370 (23), 200 (19), 177 (19), 151 (23), 109 (23),105 (38), 90 (73), 77 (39), 57 (100). – $C_{24}H_{22}N_4O_6$ (486.48): Calcd. C 64.19, H 4.56, N 11.52; found C 64.01, H 4.36, N 11.32.

4-[Aryl(4-methoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ols (5-7)

Procedure (a): A solution of **2a** (2.92 g, 0.01 mol) and resorcinol or phloroglucinol or pyrogallol (0.01 mol) in absolute ethanol (50 mL) was refluxed for 6 h. The product that was obtained on cooling was filtered and washed with boiling ethanol to give **5**-**7**.

Procedure (b): A solution of **1** (1.74 g, 0.01 mol), *p*-anisaldehyde (1.18 g, 0.01 mol) and the appropriate phenol (0.01 mol) in acetic acid (30 mL) was refluxed for 6 h. The product that was obtained was filtered and washed with boiling ethanol to give **5-7**. Yield 60, 52 and 55 %, respectively. The structure was confirmed by a comparison of IR data, mp. and TLC with that from Procedure (a).

4-[(2,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (5)

M. p: 175°C. Yield 60 % (buff powder). − IR (KBr): ν = 3418 (OH), 1600 (C=N), 1500, 1460, 1247, 1176, 1028, 758 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 229 (36) $[M\text{-pyrazolol ion}]^+$, 230 (38), 159 (8) $[\text{pyrazolol ion-Me}]^+$, 110 (100) $[\text{resorcinol ion + H}]^+$, 109 (30), 108 (47), 77 (43) $[Ph]^+$. − $C_{24}H_{22}N_2O_4$ (402.44): Calcd. C 71.63, H 5.51, N 6.96; found C 71.52, H 5.43, N 6.81.

4-[(4-Methoxyphenyl)(2,4,6-trihydroxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (6)

M. p: 188-190°C. Yield 52 % (buff powder). − IR (KBr): ν = 3444 (OH), 1605 (C=N), 1500, 1459, 1247, 1274, 1013, 755 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 420 (1) $[M+2]^+$, 293 (21) $[M\text{-phloroglucinol ion}]^+$, 292 (100) $[M\text{-(phloroglucinol ion + H)}]^+$, 245 (3) $[M\text{-pyrazolol ion}]^+$, 173 (14) $[\text{pyrazolol ion}]^+$, 126 (12) $[\text{phloroglucinol ion + H}]^+$, 77 (78) $[Ph]^+$. − $C_{24}H_{22}N_2O_5$ (418.44): Calcd. C 68.89, H 5.30, N 6.69; found C 68.77, H 5.21, N 6.55.

4-[(4-Methoxyphenyl)(2,3,4-trihydroxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (7)

M. p: 190-192°C. Yield 55 % (buff powder). − IR (KBr): ν = 3444 (OH), 1604 (C=N), 1500, 1458, 1247, 1176, 1030, 755 cm^{-1}. – ^1H NMR (300 MHz, [D$_6$] DMSO, 25 ºC, TMS): δ = 2.48 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.19 (s, 1H, O*H*), 5.59 (s, 1H, O*H*), 5.72 (s, 1H, O*H*), 5.94 (s, 1H, C*H*), 7.54 (br. s, 1H, enolic O*H*), 6.43-6.65 (m, 11H, aromatic). − MS (EI, 70 eV): m/z (%) = 415 (6) $[M-3]^+$, 246 (35), 245 (19) $[M\text{-pyrazolol ion}]^+$, 244 (36), 174 (10), 126 (94) $[\text{pyrogallol ion}]^+$, 108 (73), 77 (44) $[Ph]^+$. − $C_{24}H_{22}N_2O_5$ (418.44): Calcd. C 68.89, H 5.30, N 6.69; found C 68.76, H 5.22, N 6.56.

4-(Diarylmethyl)-3-methyl-1-phenyl-1H-pyrazol-5-ols (8-10)

These compounds were prepared from equimolar amounts of **2b** or **2c** and resorcinol or pyrogallol or 8-hydroxyquinoline (0.005 mol) in absolute ethanol (50 mL), following the procedure (a) as described above. Crystallization of the product from ethanol gave **8-10**.

4-[(2,4-Dihydroxyphenyl)(4-hydroxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (8a)

M. p. 210^{o}C. Yield 42 % (brown powder). − IR (KBr): *ν* = 3450 (OH), 1598 (C=N), 1500, 1426, 1270, 1274, 1041, 769 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 370 (11) [M-OH]$^+$, 359 (13) [M-2 OH]$^+$, 279 (13) [M-resorcinol ion]$^+$, 278 (100) [M-(resorcinol ion + H)]$^+$, 277 (48), 215 (20) [M-pyrazolol ion]$^+$, 160 (11) [pyrazolol ion-Me]$^+$,110 (46), 109 (15) [resorcinol ion]$^+$, 94 (74) [C_6H_5OH]$^+$, 77 (26) [Ph]$^+$. − $C_{23}H_{20}N_2O_4$ (388.42): Calcd. C 71.12, H 5.19, N 7.21; found C 71.00, H 5.10, N 7.09.

4-[(2,4-Dihydroxyphenyl)(4-hydroxy-3-methoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (8b)

Mp: 212^{o}C. Yield 46 % (pale brown powder). − IR (KBr): *ν* = 3419 (OH), 1602 (C=N), 1500, 1277, 1125, 757 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 418 (0.5) [M]$^+$, 309 (13) [M-resorcinol ion]$^+$, 308 (71), 185 (40), 174 (59) [pyrazolol ion + H]$^+$, 109 (7) [resorcinol ion]$^+$, 110 (16), 78 (100) [Ph + H]$^+$. − $C_{24}H_{22}N_2O_5$ (418.44): Calcd. C 68.89, H 5.30, N 6.69; found C 68.80, H 5.11, N 6.48.

4-[(4-Hydroxyphenyl)(2,3,4-trihydroxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (9a)

M. p. 110^{o}C. Yield 47 % (brown powder). − IR (KBr): *ν* = 3273 (OH), 1607 (C=N), 1498, 1250, 1171, 1021, 756 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 400 (10) [M-4]$^+$, 279 (5) [M-pyrogallol ion]$^+$, 173 (50), 174 (100) [pyrazolol ion + H]$^+$, 126 (5) [pyrogallol ion + H]$^+$, 94 (20), 77 (40) [Ph]$^+$. − $C_{23}H_{20}N_2O_5$ (404.42): Calcd. C 68.31, H 4.98, N 6.93; found C 68.10, H 4.78, N 6.80.

4-[(4-Hydroxy-3-methoxyphenyl)(2,3,4-trihydroxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (9b)

M. p. 125-126^{o}C. Yield 62 % (brown powder). − IR (KBr): *ν* = 3438 (OH), 1597 (C=N), 1500, 1365, 1278, 1029, 785 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 308 (13) [M-pyrogallol ion]$^+$, 174 (47), 124 (23) [pyrogallol ion - H]$^+$, 109 (20) [pyrogallol ion - OH]$^+$, 77 (100) [Ph]$^+$. – $C_{24}H_{22}N_2O_6$ (434.44): Calcd. C 66.35, H 5.10, N 6.45; found C 66.21, H 4.88, N 6.38.

7-[(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-hydroxyphenyl)methyl)quinolin-8-ol (10a)

M. p. 190^{o}C. Yield 50 % (yellow crystals). − IR (KBr): *ν* = 3247 (OH), 1602 (C=N), 1500, 1422, 1210, 731 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 411 (1) [M+3(-Me)]$^+$, 279 (18) [M-(8-hydroxyquinoline ion)]$^+$,251 (1) [M-pyrazolol ion]$^+$, 185 (52), 174 (56) [pyrazolol ion]$^+$, 145 (29) [8-hydroxyquinoline ion]$^+$, 174 (56), 93(5) [C_6H_4OH]$^+$, 77(100) [Ph]$^+$. − $C_{22}H_{21}N_3O_3$ (423.74): Calcd. C 73.74, H 5.00, N 9.92; found C 73.60, H 4.88, N 9.80.

7-[(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-hydroxy-3-methoxyphenyl)methyl)-quinolin-8-ol (10b)

M. p. 170^{o}C. Yield 50 % (yellow crystal). − IR (KBr): *ν* = 3419 (OH), 1599 (C=N), 1500, 1391, 1258, 1126, 755 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 453 (1) [M]$^+$, 452 (2) [M-1]$^+$, 331 (1) [M-C_6H_5OH]$^+$, 308 (64) [M-(8-hydroxyquinoline ion)]$^+$, 280 (37) [M-pyrazolol ion]$^+$, 174 (43), 173 (33) [pyrazolol ion] $^+$, 145 (51), 144 (17) [8-hydroxyquinoline ion] $^+$, 77 (100) [Ph]$^+$. − $C_{27}H_{23}N_3O_4$ (453.49): Calcd. C 71.51, H 5.11, N 9.27; found C 71.43, H 5.00, N 9.19.

4-[(2,4-Dimethoxyphenyl)(3,4-dimethoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol(11)

To a solution of **8b** (2.09 g, 0.005 mol) and dimethyl sulphate (1.89 ml, 0.02 mol) in methanol (50 mL), sodium hydroxide solution (70%, 2 mL) was added and the mixture was refluxed for 1hr. The product obtained on cooling was crystallized from dilute ethanol to give **11**. M. p. >300ºC. Yield 40% (reddish powder). − IR (KBr): *ν* = 3450 (OH), 1638(C=N), 1206, 880, 618 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 429 (5) [M-(OMe)]$^+$, 323 (10) [M-$C_6H_3(OMe)_2$], 287(20) [M-pyrazolol ion]$^+$, 257 (10) [M-pyrazolol ion+OMe]$^+$, 207 (100), 173 (6) [pyrazolol ion]$^+$, 97 (15) [pyrazolol ion-Ph] $^+$, 77 (20) [Ph]$^+$. − $C_{27}H_{28}N_2O_5$ (460.52): Calcd. C 70.42, H 6.13, N 6.08; found C 70.30, H 6.00, N 6.17.

4-[(Aryl)(4-hydroxy-3-methoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-ols 12, 13

A mixture **2c** (1.54g, 0.005 mol) and orcinol or 2-naphthol (0.005mol) in absolute ethanol (50 mL) was refluxed for 10 h. The product obtained on cooling was crystallized from ethanol to give **12** and**13**.

4-[(2,4-Dihydroxy-6-methylphenyl)(4-hydroxy-3-methoxyphenyl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-ol (12)

M. p. 120ºC. Yield 75% (reddish powder). − IR (KBr): *ν* = 3422 (OH), 1597 (C=N), 1500, 1458, 1278, 756 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 431 (15) $[M-1]^+$, 259 (15) $[M\text{-pyrazolol ion}]^+$, 188 (100) [M-(orcinol ion+$C_6H_3OMe(OH)]^+$, 187 (25), 173 (20) $[\text{pyrazolol ion}]^+$, 123 (10) $[\text{orcinol ion}]^+$, 97 (30) $[\text{pyrazolol ion-Ph}]^+$, 82 (20) [pyrazolol ion-$(Ph+Me)]^+$, 77 (80) $[Ph]^+$. − $C_{25}H_{24}N_2O_5$ (432.47): Calcd. C 69.43, H 5.59, N 6.48; found C 69.30, H 5.47, N 6.39.

4-[(4-Hydroxy-3-methoxyphenyl)(2-hydroxynaphthalen-1-yl)methyl]-3-methyl-1-phenyl-1H-pyrazol-5-ol (13)

M. p. 203-5ºC. Yield 75 % (white powder). − IR (KBr): *ν* = 3422 (OH), 1597 (C=N), 1500, 1458, 1278, 756 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 279 (35) $[M\text{-(pyrazolol ion + H)}]^+$, 278 (33) [M-(β-naphthol ion+Me)]$^+$, 249 (16) $[M\text{-(pyrazolol ion + OMe)}]^+$, 174 (45) [pyrazolol ion+H]$^+$, 145 (18) [β-naphthol + H]$^+$, 94 (18) $[C_6H_5OH]^+$, 91 (62), 77(100) $[Ph]^+$. − $C_{28}H_{24}N_2O_4$ (452.50): Calcd. C 74.32, H 5.35, N 6.19; found C 74.21, H 5.23, N 6.10.

4-Aryl-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-c]pyrazolols 14a, b and 15a, b

A mixture of **1** (1.74 g, 0.01 mol), p-hydroxybenzaldehyde or vanilline (0.01 mol) and the appropriate phenol (0.01 mol) in acetic acid (50 mL) and conc. HCl (0.5 mL) was heated on a water bath for 2 h. The product that was obtained on cooling was filtered and washed with boiling ethanol to give ***14a**, **b** and **15a**, **b***.

4-(4-Hydroxyphenyl)-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-c]pyrazol-7-ol (14a)

M. p. >300ºC. Yield 80% (brown powder). − IR (KBr): *ν* = 3382 (OH), 1612 (C=N), 1500, 1511, 1425, 1277, 1057, 829 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 359 (17), 358 (62), 355 (50) $[M+3(-Me)]^+$, 341(30) $[M-(Me+OH)]^+$, 279 (25) $[M-(Me+Ph)]^+$, 216 (12), 185 (33), 158 (9), 77 (100) $[Ph]^+$. − $C_{23}H_{18}N_2O_3$ (370.40): Calcd. C 74.58, H 4.90, N 7.56; found C 74.44, H 4.79, N 7.49.

4-(4-Hydroxy-3-methoxyphenyl)-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-]pyrazol-7-ol (14b)

M. p.>300ºC. Yield 75 % (buff powder). − IR (KBr): *ν* = 3405(OH), (1609), (C=N), 1500, 1465, 1159, 721 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 397 (39) $[M-3]^+$, 292 (33) $[M-(Ph+2OH)]^+$, 160 (67), 103 (67), 60(100), 87 (33), 77 (72) $[Ph]^+$. − $C_{24}H_{20}N_2O_4$ (400.43): calcd. C 71.99, H 5.03, N 7.00; found C 71.81, H 4.94, N 6.91.

4-(4-Hydroxyphenyl)-3-methyl-1-phenyl-1,4-dihydrochromeno[2,3-c]pyrazol-7,8-diol (15a)

M. p. 145ºC. Yield 70 % (brown powder). − IR (KBr): *ν* = 3443 (OH), 1597 (C=N), 1500, 1367, 1273, 1173, 755 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 388 (50) $[M+2]^+$, 389 (10) $[M+3]^+$, 149 (50), 131 (70), 97 (70), 73 (100). − $C_{23}H_{18}N_2O_4$ (386.40): Calcd. C 71.49, H 4.70, N 7.25; found C 71.37, H 4.62, N 7.13.

4-(4-Hydroxy-3-methoxyphenyl)-3-methyl-1-phenyl-1,4 dihydrochromeno[2,3-c]pyrazol-7,8-diol (15b)

M. p. >300ºC. Yield 75% (buff powder). − IR (KBr): *ν* = 3384 (OH), 1609 (C=N), 1517, 1428, 1076, 758 cm^{-1}. – ^1H NMR (200 MHz, $[D_6]$ DMSO, 25 ºC, TMS): δ = 2.49 (s, 3H, CH_3), 3.42 (s, 3H, OCH_3), 5.54 (s, 1H, 4-H), 5.56 (s, 1H, OH), 6.04 (s, 1H, OH), 6.15-7.62 (m, 10H, aromatic), 7.88 (s, 1H, OH). – MS (EI, 70 eV): m/z (%) = 416 (55) $[M]^+$, 376 (57), 355 (57) $[M-(3OH+Me)]^+$, 185 (78), 91 (66), 78 (66). − $C_{24}H_{20}N_2O_5$ (416.43): Calcd. C 69.22, H 4.84, N 6.73; found C 69.10, H 4.70, N 6.62.

7-(4-Methoxyphenyl)-8-methyl-10-phenyl-pyrazolo[5,4-b]benzo[h]chromen-4-ol (17a)

A solution of **2a** (0.01 mol) and 1,5-dihydroxynaphthalene (0.01 mol) in absolute ethanol (50 mL) and 2-3 drops of conc. HCl was refluxed for 6 h. The product that was obtained on cooling was crystallized from ethanol to give **17a**. M. p. 167ºC. Yield 65 % (violet powder). − IR (KBr): *ν* = 3438(OH), 1597(C=N), 1500, 1248, 1176, 1030, 755 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 433 (27) $[M-1]^+$, 299 (18), 292 (36), 174 (68), 131 (41), 91 (100), 77 (32) $[Ph]^+$. − $C_{28}H_{22}N_2O_3$ (434.49): Calcd. C 77.40, H 5.10, N 6.45; found C 77.29, H 5.01, N 6.37.

7-(4-Hydroxy-3-methoxyphenyl)-8-methyl-10-phenyl-pyrazolo[5,4-b]benzo[h]chromen-4-ol (17b)

This compound was obtained from equimolar amounts of **2c** and 1,5-dihydroxynaphthalene (5 mmol), following the procedure described above for the synthesis of **17a**. The product was crystallized from ethanol to give **17b**. M. p. 242ºC. Yield 68% (violet powder). − IR (KBr): *v* = 3426(OH), 1628 (C=N), 1598 (C=C), 1500, 1376, 1273, 1031, 756 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 452 (6) $[M+2]^+$, 358 (7) $[M-(Me+Ph)]^+$, 308 (43) $[M-(1.5\text{-dihydroxynaphthaline ion})]^+$, 200 (12), 174 (69), 160 (13), 105 (40), 91 (68), 77 (100) $[Ph]^+$, 51 (50). − $C_{28}H_{22}N_2O_4$ (450.49): Calcd. C 74.65, H 4.92, N 6.22; found C 74.51, H 4.80, N 6.10.

3-[(3-Methyl-1-phenyl-pyrazol-ol-4yl)arylmethyl)]-*7-(aryl)-8-methyl-10-phenyl pyrazolo[5,4-b]benzo[h]chromen-4-ols 18a-c*

A solution of **2a** or **2b** or **2c** (0.01 mol) and 1,5-dihydroxynaphthalene (0.005 mol) in absolute ethanol (50 mL) and 2-3 drops of conc. HCl was refluxed for 6 h. The product that was obtained on cooling was crystallized from ethanol to give **18a-c**.

Compound ***18a****:* M. p. 244 ºC. Yield 70 % (violet powder). − IR (KBr): *v* = 3420 (OH), 1606 (C=N), 1500, 1372, 1277, 1109, 782 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 728 (16) $[M+2]^+$, 359 (13), 300 (22), 292 (54), 174 (51) $[\text{pyrazolol ion}]^+$, 185 (30), 160 (16), 77 (100) $[Ph]^+$. − $C_{46}H_{38}N_4O_5$ (726.82): Calcd. C 76.02, H 5.27, N 7.7; found C 75.94, H 5.15, N 7.61.

Compound ***18b***: M. p. 215ºC. Yield 65 % (violet powder) − IR (KBr): *v* = 3417 (OH), 1600 (C=N), 1595 (C=C), 1500, 1380, 1232, 1170, 754 cm^{-1}. – ^1H NMR (300 MHz, $[D_6]$ DMSO, 25 ºC, TMS): δ = 2.31 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 5.44 (s, 1H, 7-H), 5.74(s, 1H, CH), 6.63-7.68 (m, 22H, aromatic), 7.82(br. s, 1H, enolic OH), 7.92 (s, 1H, OH), 8.60 (s, 1H, O*H*), 8.63 (s, 1H, O*H*). – MS (EI, 70 eV): m/z (%) = 685 (22) $[M+2(-Me)]^+$, 278 (35), 174 (60) $[\text{pyrazolol ion}]^+$, 185 (32), 91 (70), 77 (100) $[Ph]^+$. − $C_{44}H_{34}N_4O_5$ (698.76): Calcd. C 75.63, H 4.90, N 8.02; found C 75.52, H 4.83, N 7.91.

Compound ***18c****:* M. p. >300 ºC. Yield 70% (violet powder). − IR (KBr): *v* = 3419 (OH), 1599 (C=N), 1500, 1461, 1274, 1149, 765 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 759 (6) $[M+1]^+$, 731 (8) $[M-(2Me+H)]^+$, 647 (7), 308

(38), 185 (28), 174 (41) [pyrazolol ion]$^+$, 77 (100) [Ph]$^+$. − $C_{46}H_{38}N_4O_7$ (758.82): Calcd. C 72.81, H 5.05, N 7.38; found C 72.69, H 4.94, N 7.28.

Synthesis of Mannich bases 20-22

A solution of **2b** or **2c** (0.01 mol), formalin (37%, 0.03 mol) and the appropriate *sec*. amine (0.01mol) in absolute ethanol (50 mL) was refluxed for 10 h. The product that was obtained on cooling was crystallized from ethanol to give **20-22**.

4-[(4-Hydroxy-3-(piperidin-1-ylmethyl)benzylidene]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (20)

M. p. 65-8ºC. Yield 50 % (white crystals). − IR (KBr): ν = 3434 (OH), 2918, 2849, 1629 (C=O), 1463, 1378, 720 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 378 (1) [M+3]$^+$, 377 (1) [M+2]$^+$, 99 (12), 85 (41), 57 (100) [$CH_2N(CH_2)_2$]$^+$. − $C_{23}H_{25}N_3O_2$ (375.46): Calcd. C 73.57, H 6.71, N 11.19; found C 73.43, H 6.60, N 11.08.

4-[3-((Dimethylamino)methyl)-4-hydroxy-5-methoxybenzylidene]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (21*)*

M. p. 75ºC. Yield 55% (white crystals). − IR (KBr): ν = 3433 (OH), 2918, 2849, 1627 (C=O), 1597, 1463, 1378, 719 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 365 (0.5) [M]$^+$, 352 (1) [M +2(-Me)]$^+$, 337 (1) [M–(Me+OH)]$^+$, 308 (1) [M–$CH_2N(Me)_2$]$^+$, 181 (1) [M–pyrazolone ion]$^+$, 71 (60), 59 (1) [$CH_2N(Me)_2$]$^+$, 57 (100) [$CH_2N(CH_2)_2$]$^+$. − $C_{21}H_{23}N_3O_3$ (365.43): Calcd. C 69.02, H 6.34, N 11.50; found C 68.90, H 6.26, N 11.40.

4-[(4-Hydroxy-3-methoxy-5-(morpholinomethyl)benzylidene]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (22)

M. p. 85-7ºC. Yield 75% (pale brown crystals). − IR (KBr): ν = 3434 (OH), 2918, 2849, 1629 (C=O), 1463, 1378, 719 cm^{-1}. – MS (EI, 70 eV): m/z (%) =406 (5) [M-1]$^+$, 340 (10) [M–(OH+Me)]$^+$, 281 (50), 207(100), 135 (25), 77 (10). − $C_{23}H_{25}N_3O_4$ (407.46): Calcd. C 67.80, H 6.18, N 10.31; found C 67.69, H 5.97, N 10.20.

N,N'-Di(aryl-3-methyl-1-phenyl-5-pyrazolone-4-ylmethyl)-4,4''-trimethylenedipiperidines (24a, b)

A solution of **2a** (2.92 g, 0.01 mol) or **2b** (2.78 g, 0.01 mol) and 4,4’-trimethylenedipiperidine (**23**) (1.05 g, 0.005 mol) in absolute ethanol

(50 mL), was refluxed for 2 h. After standing at r. t. for 24 h, the reaction mixture was concentrated and the product was filtered and crystallized from ethanol to give **24a**, **b**.

N,N'-Di(4-methoxyphenyl-3-methyl-1-phenyl-5-pyrazolone-4-ylmethyl)-4,4''-trimethylenedipiperidine (24a)

M. p. 200-201^{o}C. Yield 57 % (yellow crysrals). − IR (KBr): ν = 1642 (C=O), 1598(C=N), 1502, 1360, 1246, 1036, 791 cm^{-1}. – ^{1}H NMR (300 MHz, [D_6] DMSO, 25 ºC, TMS): δ = 1.11-1.15 (m, 4H, 1-H_2, 3-H_2 of propane), 1.19 (m, 2H, 2 x 4-H of piperidine), 1.66-1.71 (m, 2H, 2-H_2 of propane), 2.15 (s, 6H, 2 x CH_3), 2.69-2.73 (m, 8H, 2 x 3–H_2, 5-H_2 of piperidine), 2.73-2.77 (m, 8H, 2 x 2–H_2, 6-H_2 of piperidine), 3.14 (d, 2H, 2 x 4-H of pyrazolone), 3.68 (s, 6H, 2 x OCH_3), 4.58 (d, 2H, 2 x C*H*), 6.74-7.96 (m, 18H, aromatic). − ^{13}C NMR (200 MHz, [D_6] DMSO): δ = 22.57 (CH_3), 32.06 (C-4 of piperidine), 37.93 (C-2 of propane), 42.24 (C-1, C-3 of propane), 43.42 (C-3, C-5 of piperidine), 44.96 (C-2, C-6 of piperidine), 49.79 (C-4 of pyrazolone), 52.80 (*C*H), 64.39 (OCH_3), 111.78, 122.50, 128.45, 132.26, 137.66, 148.15, 155.21, 166.26 (all Ar-*C*), 150.25 (C-3 of pyrazolone), 166.63 (C=O). − MS (EI, 70 eV): *m/z* (%) = 798 (7) [M+3]$^+$, 799 (17), 346 (21), 292 (31), 261 (17), 207 (14), 186 (21), 106 (24), 77 (100) [Ph]$^+$. − $C_{49}H_{58}N_6O_4$ (795.02): Calcd. C 74.03, H 7.35, N 10.57; found C 73.81, H 7.12, N 10.29.

N,N'-Di(4-hydroxyphenyl-3-methyl-1-phenyl-5-pyrazolone-4-ylmethyl)-4,4''-trimethylenedipiperidine (24b)

M. p. 183-185^{o}C. Yield 48 % (reddish powder). − IR (KBr): ν = 3435 (OH), 1640 (C=O), 1606 (C=N), 1510, 1370, 1265, 1033, 759 cm^{-1}. – MS (EI, 70 eV): m/z (%) = 722 (26) [(M+1)-(2 Me+OH)]$^+$, 278 (100), 277 (42), 174 (26), 98 (37), 91 (52), 77 (79) [Ph]$^+$. − $C_{47}H_{54}N_6O_4$ (766.97): Calcd. C 73.60, H 7.10, N 10.96; found C 73.47, H 6.98, N 10.79.

Pharmacology

Materials and Methods

Antioxidant screening; ABTS method (Gazzar, et. al., 2009)

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 (20µL 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:

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

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

Bleomycin-dependent DNA damage

The assay was performed according to **(Aeschbach, et al., 1981)** and Chan & Tang **(Chan & Tang 1996)**, 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 ^oC for 1 h, and 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^oC 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.

Reagents for lymphocyte transformation assay

Heparinized peripheral venous blood was obtained from healthy volunteers from the blood bank of Mansoura University Hospital; Ficoll/Hypaque obtained from Amersham Pharmacia, Uppsala, Sweden; phytohaemagglutinin (PHA) obtained from Difco, Detroit, MI, USA; Concanvalin A (ConA) obtained from Merck, Germany; Hank's balanced salt solution (HBSS); foetal calf serum (FCS); glutamine; HEPES (N-2-hydroxyethylpiperazine-N`-2-ethanesulfonic acid)-buffer and RPMI-1640 medium obtained from Gibco BRL, Life Technologies, Pailsey, Scotland; crystalline penicillin G and streptomycin obtained from El-Nile Pharmaceutical Co., Cairo, Egypt.

Standards (+ve) *Echinacea Purpurea* extract (Immulone ®) obtained from Sekem Pharmaceutial Co., Cairo, Egypt. Levamisole (Ketrax ®) obtained from Elkahira Pharmaceutial Co., Cairo, Egypt (manufactured under license from AstraZeneca, Wilmington, Delaware, USA). Standard (-Ve) Cyclophosphamide (Endoxan ®) obtained from ASTA Medica AG, Frankfurt, Germany. Cyclosporin (Sandimmune Neoral ®) obtained from Novartis Pharma, Switzerland.

A) Separation of Peripheral Blood Lymphocytes (PBL)

Lymphocytes were separated from peripheral human venous blood by Ficoll/Hypaque gradient technique. For each sample, 5 ml of heparinized blood was diluted with equal volume of Hank's balanced salt solution (HBSS) in a sterile plastic centrifuge tube. Diluted blood (6 ml) was carefully overlaid on 4 ml Ficoll/Hypaque solution gradient without allowing the solution to become mixed by keeping the pipette against the tube wall 5-10 mm above the fluid meniscus. The tube was centrifuged at 1200 rpm at room temperature. The lymphocytes were localized as a whitish layer on the upper meniscus of the gradient solution. Using a fine pasteur pipette, the zone containing lymphocytes was taken and washed twice in HBSS (10 min at 1200 rpm). The residue is a buffy coat of polymorphonuclear leucocytes (PMNLs).

B) Lymphocyte transformation assay

The viable lymphocytes were adjusted to a concentration of 2 x 10^6 cells/ml in RPMI-1640 medium supplemented with 600 µl penicillin, 0.1 ml streptomycin, 1% glutamine, 25% HEPES-buffer, and 20% foetal calf serum (FCS). The lymphocytes were plated into 96-well tissue culture plates (or Ependorff tubes). The test solution (100µl) in DMF (100 µl/ml) and 20 µg of the mitogen (PHA) were added to each well. Cell cultures were incubated at 37 °C in 5% CO_2 atmosphere for 72 hrs, during which the mitogen produced its maximal effect on DNA synthesis. After culture, cell films were stained by Giemsa stain and the average count of percentage of transformed (proliferated) blasts was determined.

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تشييد بعض المركبات الفينولية المحتوية على مجموعة بيرازول
ونشاطها المضاد للأكسدة

السيد محمد عفصة – ايمان محمد كشك – سها مصطفى عبد المجيد – فريد بدرية – أميرة اسماعيل عامر
كلية العلوم وكلية الصيدلة – جامعة المنصورة

أمكن تشييد عدد من المركبات الفينولية المحتوية على مجموعة بيرازول بتفاعل مركبات ٤ – أريلدين بيرازولون مع عدد من مركبات الفينول تحت ظروف كيميائية مختلفة. كما أمكن تشييد عدد من قواعد مانش الفينولية المحتوية على مجموعة بيرازول بتفاعل مانش على المركبات الفينولية المحتوية على مجموعة بيرازول. و تبين أن عدد من المركبات الجديدة تتميز بنشاط واضح كمضادات للأكسدة.