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Synthesis and antioxidant evaluation of some new 2-benzoylamino-5-hetaryl-1,3,4-oxadiazoles

Samir Bondock[1,2] · **Shymaa Adel**[2] · **Hassan A. Etman**[2]

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Abstract A series of new functionalized 2-benzoylamino-5-hetaryl-1,3,4-oxadiazoles were efficiently synthesized via the reaction of the versatile key intermediates, 2-benzoylamino-5-cyanomethyl-1,3,4-oxadiazole (**1**) and *N*-(5-(5-amino-3-phenylamino)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)-benzamide (**13**), with some appropriate electrophilic reagents. The structures of the newly synthesized compounds were established on the basis of elemental analyses, spectral data, and by alternative synthesis wherever possible. The mechanisms of the studied reactions are discussed. Also, we evaluate the antioxidant activity of some representative examples of the newly prepared compounds. Among the synthesized compounds, 2-benzoylamino-5-cyanomethyl-1,3,4-oxadiazole (**1**) and *N*-(5-(7-methyl-5-oxo-2-(phenylamino)-4,5-dihydropyrazolo[1,5-*a*]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**17**) showed excellent antioxidant activity and exhibited high protection against DNA damage induced by the Bleomycin iron complex.

Keywords 1,3,4-Oxadiazole · Pyrazole · Pyrazolo[1,5-*a*]pyrimidine · Imidazo[1,2-*b*]pyrazole · Antioxidant activity

Introduction

1,3,4-Oxadiazole constitutes an important scaffold for the development of new drugs [1]. Compounds having 1,3,4-oxadiazole skeletons possessed a wide range of therapeutic activities like antifungal [2], antioxidant [3–5], antimicrobial [6], antitubercular [7], anti-inflammatory [8, 9], antimalarial [10], antihypoglycemic

✉ Samir Bondock
Bondock@mans.edu.eg

[1] Chemistry Department, Faculty of Science, King Khalid University, Abha 9004, Saudi Arabia

[2] Chemistry Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt

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[11], and anticancer [12–14] activities as well as potential antihypertensive agents [15], antiviral [16] and anticonvulsant [17] properties. The pharmacological activity of 1,3,4-oxadiazoles can be ascribed to the presence of toxophoric –N=C–O– linkage which may react with the nucleophilic centers of the microbial cell [18]. They have also received interest in medicinal chemistry as surrogates (bioisosteres) for carboxylic acids, esters and carboxamides [19]. On the other hand, the heterocyclic moieties including pyrazoles [20], thiophenes [21], pyrazolo[1,5-*a*]pyrimidines [22] and imidazo[1,2-*b*]pyrazoles [23] have received considerable interest owing to their wide spectrum of biological activities. Merging of these two bioactive components; 1,3,4-oxadiazole and heterocyclic moiety, in the same molecule is a fascinating approach to discover novel potent drugs, due to the possible synergistic effect.

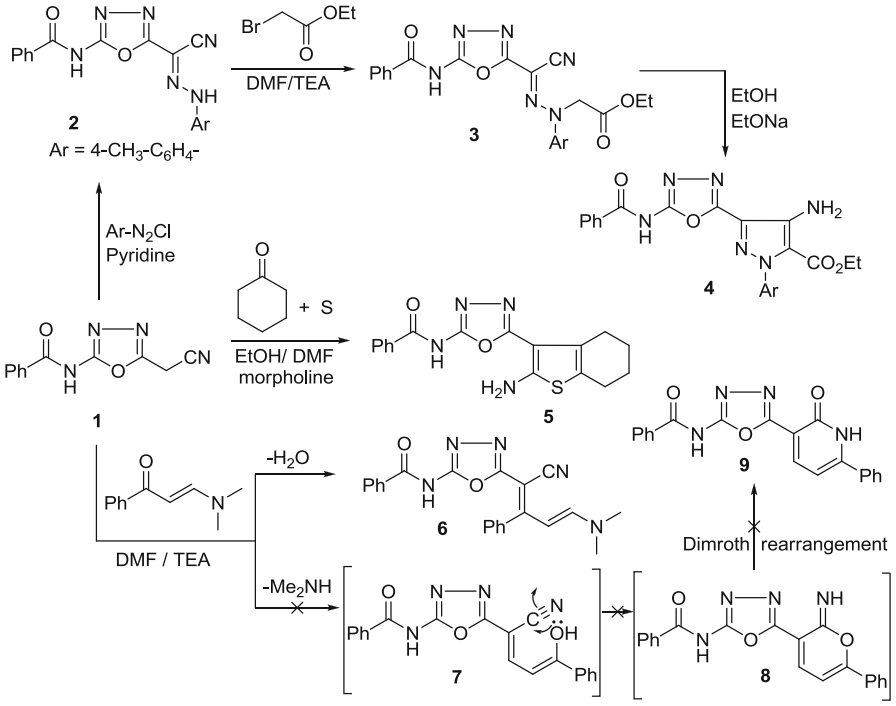
In view of the above-mentioned facts and in continuation of our ongoing program [24–32] aim to find a new class of biheterocycles with potential chemotherapeutic activities, we planned to synthesize and study the antioxidant properties of hitherto unreported 2-benzoylamino-5-hetaryl-1,3,4-oxadiazoles.

Results and discussion

Chemistry

The synthetic pathway to achieve the target compounds is depicted in Schemes 1, 2, 3 and 4. The key intermediate 2-benzoylamino-5-cyanomethyl-1,3,4-oxadiazole (**1**) was prepared by cyclodesulfurization of 1-cyanoacetyl-4-benzoylthiosemicarbazide with mercuric oxide in boiling ethanol as previously reported by us [12]. It is well known that the reaction of hydrozonyl nitrile with α-halocarbonyl compounds represents the simplest method to obtain the 4-aminopyrazoles [33]. In this context, we investigated the reaction of **1** with arene diazonium salt and α-bromoester. Thus, coupling of **1** with *p*-tolyl diazonium chloride in pyridine at 0 °C gave the hydrozonyl nitrile derivative **2**.

Alkylation of hydrazonyl nitrile **2** with ethyl bromoacetate in boiling dimethylformamide containing a catalytic amount of triethylamine afforded a single product identified as 2-(2-((5-benzamido-1,3,4-oxadiazol-2-yl)(cyano)methylene)-1-*p*-tolylhydrazinyl) acetate (**3**). The base catalyzed Thorpe-Ziegler cyclization of **3** led to the formation of ethyl 4-amino-3-(5-benzamido-1,3,4-oxadiazol-2-yl)-1-*p*-tolyl-1*H*-pyrazole-5-carboxylate (**4**). Compounds **3** and **4** were established on the basis of microanalyses and spectral data. The IR spectrum of **4** showed absorption bands at 3345–3285, 3197, 1739, 1703 cm^{-1} due to NH_2, NH, ester C=O, and amidic C=O function, respectively. Its ^{1}H–NMR spectrum revealed triplet and quartet signal at δ 1.26 and 4.35 ppm due to the ethoxy protons besides three singlet signals at 2.32, 6.35, and 12.06 due to CH_3, NH_2, and NH protons, and multiplet signal around 7.16-8.12 ppm owing to nine aromatic protons. The ^{13}C–NMR spectrum of **4** revealed 18 carbon types, the most important signals being displayed at δ 14.2, 20.7, 59.8, 160.4, and 164.8 ppm due to two methyl carbons, OCH_2, and two carbonyl carbons, respectively. Its mass spectrum showed a molecular ion peak at $m/z = 432$ (M^+) which fits with a molecular formula ($C_{22}H_{20}N_6O_4$).

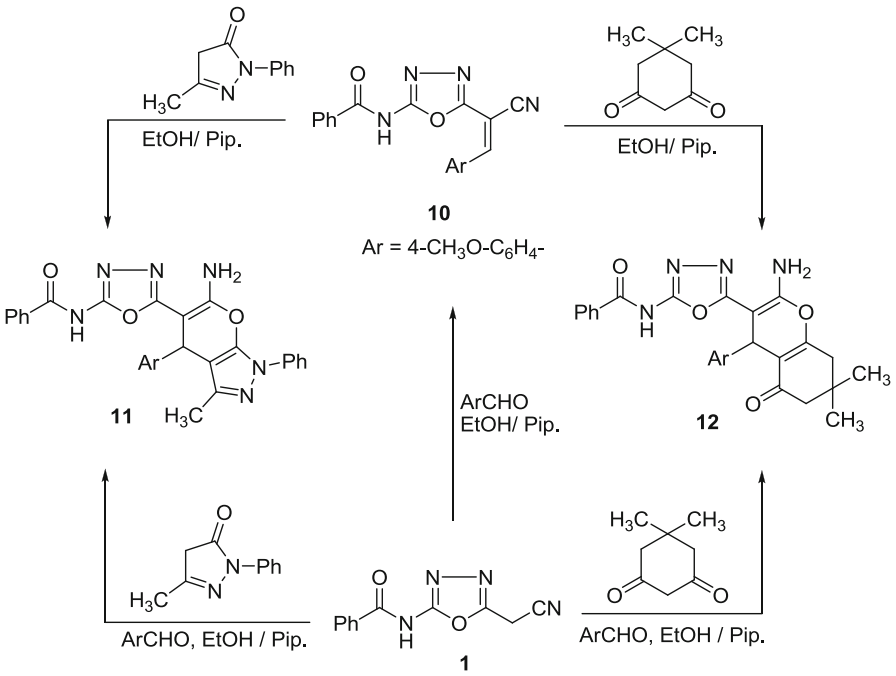


Scheme 1 Synthetic route to pyrazolyl-, tetrahydrobenzothienyl- and acroyl oxadiazoles **4**–**6**

The Gewald reaction of **1** with elemental sulfur and cyclohexanone in a warming mixture of ethanol and dimethylformamide (1:1) containing morpholine as a basic catalyst gave *N*-(5-(2-amino-4,5,6,7-tetrahydrobenzo[*b*] thiophen-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**5**). The IR spectrum of **5** displayed absorption bands at 3446–3388, 3195, 1675 cm^{-1} due to NH_2, NH, and amidic C=O functions, respectively. Also, its mass spectrum showed the molecular ion peak at $m/z = 340$ (M^+), corresponding to the molecular formula ($C_{17}H_{16}N_4O_2S$).

The reaction of enaminones with activated nitriles has been reported as an efficient synthetic route to attain 2-pyridones [34]. In this context, we investigated the reaction of activated nitrile moiety of **1** with enaminone. Thus, treatment of **1** with enaminone, *E*-3-(dimethylamino)-1-phenylprop-2-en-1-one [35], in boiling dimethylformamide containing a catalytic amount of triethylamine afforded, a single product, identified as *N*-(5-(1-cyano-4-(dimethylamino)-2-phenylbuta-1,3-dienyl)-1,3,4-oxadiazol-2-yl)benzamide (**6**) instead of *N*-(5-(2-oxo-6-phenyl-1,2-dihydropyridin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**9**) as expected (Scheme 1). The identity of structure **6** was based on the IR spectrum which showed absorption bands at 3222, 2213, 1678, 1625 cm^{-1} due to NH, nitrile, amidic C=O, and C=C functions, respectively. Its mass spectrum showed the molecular ion peak at $m/z = 385$ (M^+) corresponding to the molecular formula ($C_{22}H_{19}N_5O_2$).

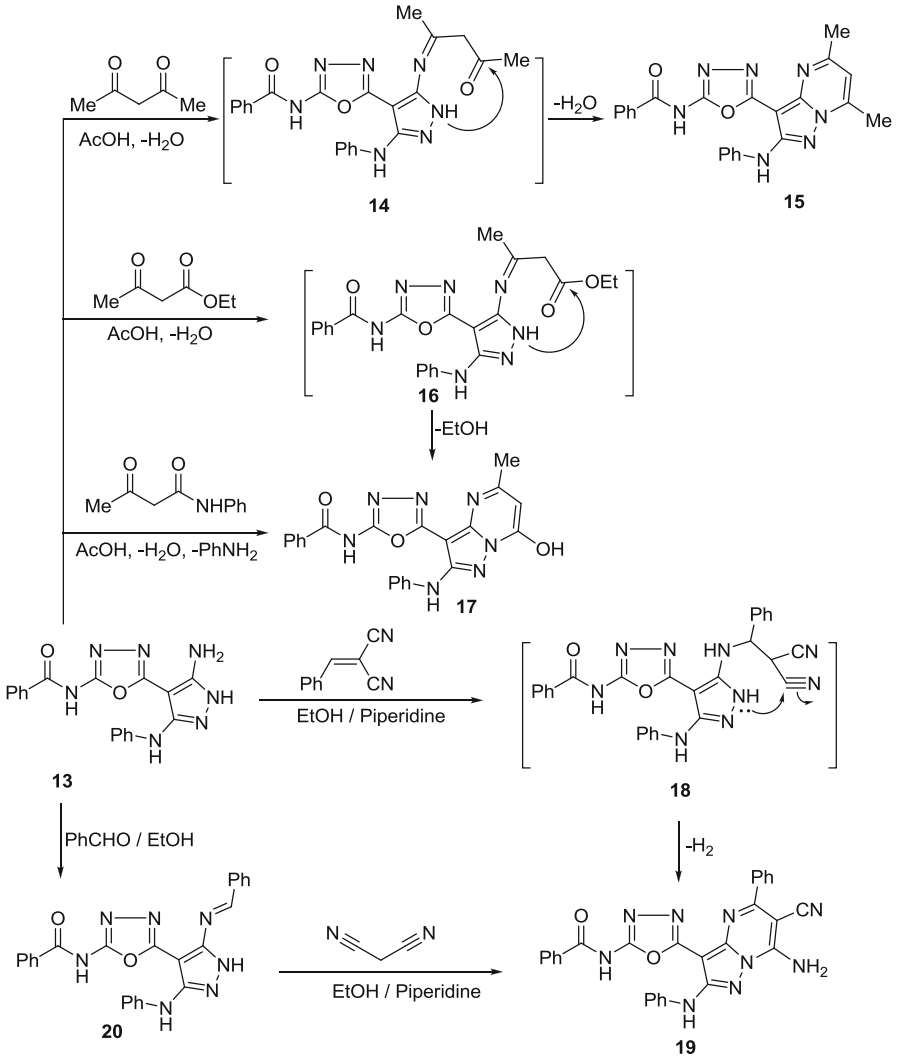
We next explored the synthetic use of **1** to obtain some novel pyrano [2,3-*c*]pyrazole and chromene based 1,3,4-oxadiazole. Thus, the Knoevenagel



Scheme 2 Synthetic route to Pyrano[2,3-c]pyrazole **11** and Chromene **12** bearing oxadiazole

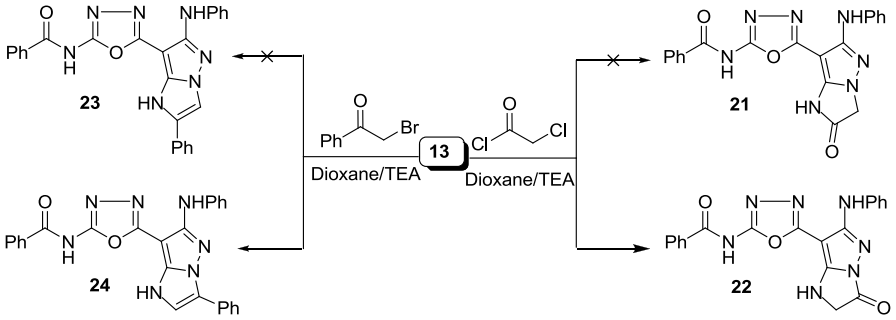
condensation of **1** with *p*-anisaldehyde in refluxing ethanolic triethylamine solution furnished the cyanoolefin derivative **10** [12]. Treatment of cyanoolefin **10** with each of 1-phenyl-3-methyl-2-pyrazolin-5-one and 5,5-dimethyl-1,3-cyclohexanedione in refluxing ethanol containing a catalytic amount of piperidine afforded the respective pyrano[2,3-*c*]pyrazole **11** and chromene **12** derivatives (Scheme 2). Compounds **11** and **12** were unambiguously synthesized by an alternative route involving the one-pot three components condensation reaction of **1** with, each of, *p*-anisaldehyde and 1-phenyl-3-methyl-2-pyrazolin-5-one, *p*-anisaldehyde and 5,5-dimethyl-1,3-cyclohexanedione, in refluxing ethanolic piperidine solution.

The analytical and spectral data are consistent with the structure proposed for compounds **11** and **12**. In general, the IR spectra of these products revealed absorption bands at 3451–3418 and 3380–3324 cm^{-1} for NH_2 group and lack of absorption bands for CN group. The mass spectra of **11** and **12** showed the molecular ion peak at *m/z* 520 (M^+, 12.5 %) and 486 (M^+, 8 %), respectively. The ^{1}H–NMR spectrum of **11** displayed four singlet signals at δ 2.32, 3.83, 4.84, 12.31 ppm assignable to methyl, methoxy, pyran-H_4, amidic NH protons, respectively, besides a complex multiplet at δ 7.12–8.12 ppm due to the amino and aromatic protons. Formation of compounds **11** and **12** could be explained via a mechanism starting with the Michael addition of an active methylene group of pyrazolone and dimedone to the ylidenic bond in cyanoolefin **10** to give an acyclic intermediate, which cyclized in situ by the nucleophilic attack of the enolic (OH) group on the cyanocarbon, followed by tautomerization to give the final products.



Scheme 3 Synthetic route to pyrazolo[1,5-*a*]pyrimidines **15**, **17** and **19** bearing oxadiazole

5-Aminopyrazoles are versatile precursor and have been extensively used as building blocks for the synthesis of several polysubstituted condensed pyrazoles of potent pharmacological activity [36]. It was thus of interest to investigate the reactivity of the second key intermediate *N*-(5-(5-amino-3-phenylamino)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)-benzamide (**13**) [12] towards a variety of bifunctional electrophilic reagents. The general literature procedure [37] for the synthesis of pyrazolo[1,5-*a*]pyrimidines involves cyclocondensation of aminopyrazoles with 1,3-bielectrophilic reagents. Thus, cyclocondensation reaction of compound **13** with acetylacetone in boiling acetic acid produced a single product



Scheme 4 Synthetic route to imidazo[1,2-*b*]pyrazoles **22** and **24** bearing oxadiazoles

formulated as *N*-(5-(5,7-dimethyl-2-(phenylamino)pyrazolo[1,5-*a*]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**15**).

The structure **15** was confirmed on the basis of elemental analysis and spectral data. The IR spectrum displayed absorption bands at 3332, 3265 and 1664 cm^{-1} due to two NH and an amidic C=O functions. The ^{1}H–NMR spectrum (DMSO-d_6) exhibited three sharp singlet signals at δ 2.61, 2.75 and 6.95 ppm assignable to two unequivalent methyl protons and fused pyrimidine (H_5) protons, respectively, besides a multiplet signal at δ 7.05–7.94 ppm region owing to aromatic protons, and two broad singlet signals, exchangeable with D_2O, at δ 10.65 and 11.36 ppm due to two NH protons. The mass spectrum showed a molecular ion peak at $m/z = 425$, corresponding to molecular formula ($C_{23}H_{19}N_7O_2$).

Analogously, compound **13** was reacted with ethyl acetoacetate in boiling acetic acid to give an isolable product, formulated as *N*-(5-(7-hydroxy-5-methyl-2-(phenylamino)pyrazolo[1,5-*a*]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**17**). Structure **17** was elucidated by elemental analysis, spectral data, and an alternative synthesis route. Thus, treatment of **13** with acetoacetanilide in boiling acetic acid gave product identical in all aspects (m.p., mixed m.p., and spectra) with **17**.

The formation of compound **17** is assumed to proceed via initial attacks of the exocyclic amino group of **13** on the keto group of ethyl acetoacetate followed by elimination of water to give the non-isolable intermediate **16** that underwent intramolecular cyclization via elimination of ethanol molecule.

Moreover, treatment of **13** with α-cyanocinnamonitrile in boiling ethanol containing a catalytic amount of piperidine, under reflux, gave isolable product evidence by mechanism which could be formulated *N*-(5-(7-amino-6-cyano-5-phenyl-2-(phenylamino)pyrazolo[1,5-*a*]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**19**).

The reaction seems to proceed through Michael addition reaction of the exocyclic amino group to β-carbon of cinnamonitrile to give intermediate **18** which underwent intramolecular cyclization via the addition of the endocyclic NH group to the nitrile functional group and subsequent oxidation to give the final product **19**. More evidences for structure **19** came from its independent synthesis, treatment of **20**, which was prepared via condensation of **13** with benzaldehyde, with malononitrile

in boiling ethanol containing a catalytic amount of piperidine gave a product identical in all aspects (m.p., mixed m.p., and spectra) with **19** (Scheme 3).

Literature survey revealed that there have been few reports on the synthesis of imidazo[1,2-*b*]pyrazoles [23, 38]. The interest in the synthesis of azapentalene ring system is attributed to the fact that they are useful in both theoretical chemistry and material sciences [39]. Thus, from a structure–activity viewpoint, the insertion of 1,3,4-oxadiazole moiety into the azapentalene ring system may offer derivatives with enhanced or new utility.

In this context, we studied the regioselectivity of the cyclocondensation of 5-aminopyrazole with each of chloroacetyl chloride and phenacycl bromide as a possible synthetic route to attain imidazo[1,2-*b*]pyrazoles. Thus, cyclocondensation of **13** with chloroacetyl chloride in dioxane containing a catalytic amount of triethylamine at room temperature afforded a single product for which two probable isomeric structures were considered **21** and **22** (Scheme 4). The structure **21** was ruled out on the basis of its analytical and spectral data. The structure **22** was elucidated for the reaction product where, its ^{1}H–NMR spectrum (DMSO-d_6) showed a doublet signal at δ 4.05 ppm ($J = 6.6$ Hz) assignable to a CH_2 protons attached to NH of fused pyrazole ring, besides a multiplet signal at δ 7.32–7.93 ppm due to aromatic protons and three broad singlet signals at δ 10.25, 11.50 and 12.36 ppm due to three NH protons. The IR spectrum showed the presence of absorption bands at 1690 and 1663 cm^{-1} due to cyclic C=O and amidic C=O functions, respectively. Moreover, its mass spectrum showed the molecular ion peak at $m/z = 401$.

Similarly, the reaction of **13** with phenacyl bromide in dioxane, under reflux, in the presence of triethylamine afforded a product of a molecular formula ($C_{26}H_{19}N_7O_2$). Two theoretically possible structures were considered (*cf.* structure **23** and **24**) (Scheme 4). The structure **24** was considered most likely than **23** based on its spectroscopic data. The IR spectrum of **24** showed absorption bands at 3330–3160 cm^{-1} region due to three NH functions, at 1661 cm^{-1} characteristic to amidic C = O function. Its ^{1}H NMR spectrum (DMSO-d_6) displayed a doublet signal at δ 6.24 ppm ($J = 6.6$ Hz) assignable to cyclic methine proton adjacent to NH of fused pyrazole, a multiplet signal at δ 7.17–7.87 ppm due to aromatic protons and three broad singlet signals at δ 10.25, 11.37 and 12.66 ppm due to three NH protons. The mass spectrum showed a molecular ion peak at $m/z = 461$ (M^+), corresponding to a molecular formula ($C_{26}H_{19}N_7O_2$).

Biological evaluation

Antioxidant activity by means of ABTS method

The antioxidant activity of the newly synthesized compounds was evaluated by the ABTS antioxidant activity method as described previously by Lissi and coworkers [40]. Some of the 5-hetaryloxadiazoles exhibited an antioxidant effect as shown in Table 1. The results of antioxidant activity of 2-benzoylamino-5-hetaryl-1,3,4-oxadiazoles compared with the control (L-ascorbic acid) revealed that the antioxidant

Table 1 The antioxidant activity of the synthesized compounds by mean of ABTS method

Compound no.	Absorbance of samples	Compound no. % inhibition[a]
Control of ABTS	0.534	0.0
Ascorbic acid	0.052	90.26
1	0.121	77.34
2	0.277	48.12
4	0.336	37.07
5	0.528	1.12
6	0.507	5.05
11	0.483	9.55
12	0.445	16.66
15	0.449	15.91
17	0.197	63.10
19	0.340	36.32
20	0.220	58.80

[a] The % inhibition values were the mean of three replicates ± 0.02

Table 2 Pro-oxidant effects of compounds **1** and **17** on ferric Bleomycin-induced DNA damage

Compound no.	Bleomycin-dependent DNA damage Absorbance of sample
Control	0.0
Ascorbic acid	0.098 ± 0.001
1	0.101 ± 0.001
17	0.094 ± 0.001

activity of compounds **1** and **17** was found to be the highest. On the other hand, compounds **2**, **4**, **19**, and **20** showed a moderate antioxidant activity, and the rest of the investigated compounds showed a weak activity. From the structure activity relationship (SAR) point of view, the presence of pyrazole or pyrazolopyrimidine moieties at the 5-position of the 1,3,4-oxadiazole ring system enhanced the antioxidant activity. While the introduction of tetrahydrobenzothiophene or pyrano[2,3-*c*]pyrazole derivatives at the 5-position of oxadiazole diminished the antioxidant activity as in the case of compounds **5** and **11**.

The most potent antioxidant active compounds **1** and **17** were tested for Bleomycin-dependent DNA damage (Table 2). Damage of DNA in the presence of a Belomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents [41]. 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. DNA degradation is accompanied by the formation of a product similar to malondialdehyde. L-Ascorbic acid can also reduce Fe^{3+} to Fe^{2+} as a reducing agent. Table 2 shows that compound **1** and **17** have an excellent antioxidant activity and ability to protect DNA from the induced damage by Belomycin. These higher activities may

be attributed to the presence of a hydroxyl group attached to an aromatic ring in **17** or the secondary amino group in **1**, those can act as scavenging of free radicals.

Conclusion

In conclusion, the aim of the present study was to synthesize and evaluate the antioxidant activity of some novel 2-benzoylamino-5-hetaryl-1,3,5-oxadiazoles with the hope of discovering new structure leads to serve as potent antioxidant agent. Our aim has been verified by the synthesis of different groups of structure hybrids comprising basically the 1,3,4-oxadiazole fragment attached to either pyrazole, benzothiophene, pyrano[2,3-*c*]pyrazole, chromene, pyrazolo[1,5-*a*]pyrimidines or imidazo[1,2-*b*]pyrazoles counter parts. The obtained results clearly revealed that 1,3,4-oxadiazoles **1** and **17** linked to pyrazolo[1,5-*a*]pyrimidines showed excellent antioxidant activity and exhibited high protection against DNA damage induced by Bleomycin iron complex.

Experimental

The chemicals used for the synthesis of the compounds were obtained from Aldrich and Sigma Chemical Company without further purification. The solvents used were of analytical grade. Melting points were measured on an electrothermal Gallenkamp melting point apparatus. Elemental analyses were carried out at the Microanalytical Unit, Cairo University, Giza, Egypt; the results were in satisfactory agreement with the calculated values. The IR spectra were recorded in KBr disks on a Mattson 5000 FTIR spectrometer (not all frequencies are reported). The ^{1}H–NMR spectra were acquired using a Bruker WP 300 spectrometer at 300 MHz using *TMS* as an internal standard and DMSO-d_6 as solvent. The Mass spectra were performed using a Finnigan Incos 500 mass spectrometer at 70 eV. *N*-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)benzamide (**1**), *N*-(5-(1-Cyano-2-(4-methoxyphenyl)vinyl)-1,3,4-oxadiazol-2-yl) benzamide (**10**) and *N*-(5-(3-amino-5-(phenylamino)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)benzamide (**11**) were prepared according to previously reported procedure [12].

Synthesis of N-(5-[cyano(p-tolylhydrazono)methyl]-1,3,4-oxadiazol-2-yl)benz-amide (**2**) A solution of the *p*-tolyl diazonium chloride (0.002 mol) was added dropwise to a solution of compound **1** (0.46 g, 0.002 mol) in pyridine (20 mL) at 0–5 °C with stirring. The reaction mixture was stirred further for 6 h at 0 °C. The resulting solid was collected by filtration, dried and recrystallized from ethanol to give compound **2**.

Orange powder; Yield 73 %; m.p. 239–240 °C; IR (KBr): ν/cm^{-1} = 3230 (amidic NH), 3195 (NH), 2215 (CN), 1716 (CO), 1604 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 2.28 (s, 3H, CH_3), 7.19-8.06 (m, 9H, Ar–H), 12.21 (bs, 1H, amidic NH), 12.45 (s, 1H, NH-hydrazone); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 20.1 (CH_3), 111.3 (CN), 114.3 (C=N), 117.6 ($2CH_{Ar}$), 125.8 ($2CH_{Ar}$), 127.3 ($2CH_{Ar}$), 128.8 ($2CH_{Ar}$),

132.4 (CH_{Ar}), 133.5 (C_{Ar}), 136.3 (C_{Ar}), 140.5 (C_{Ar}), 145.9 (Oxadiazole-C_2), 146.5 (Oxadiazole-C_5), 164.5 (C=O). MS (EI, 70 eV) *m/z* (%): 346 (M^+, 15.6), 333 (6.2), 317 (5.6), 306 (0.4), 289 (10.3), 274 (8.2), 259 (0.9), 244 (10.4), 227 (10.3), 105 (100.0), 91 (11.6), 77 (37.8), 65 (4.3). Anal. Calcd. for $C_{18}H_{14}N_6O_2$ (346.34): C 62.42, H 4.07, N 24.27 %. Found: C 62.48, H 4.11, N 24.31 %.

Synthesis of ethyl 2-(2-((5-benzoylamino-1,3,4-oxadiazol-2-yl)(cyano) methylene)-1-p-tolylhydrazinyl)acetate **(3)** A mixture of compound **2** (0.34 g, 0.001 mol) and ethyl bromoacetate (0.16 mL, 0.001 mol) in DMF (20 mL) containing three drops of triethylamine was refluxed for 3 h. The reaction mixture was allowed to cool and then poured onto 100 mL ice-cold water. The resulting solid was collected by filtration, dried and recrystallized from ethanol to give compound **3**.

Orange powder; Yield 67 %; m.p. 260–261 °C; IR (KBr): ν/cm^{-1} = 3245 (NH), 3192 (NH), 1745 (CO, ester), 1713 (CO, amidic), 1602 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 1.23 (t, J = 7.0 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.12 (q, J = 7.0 Hz, 2H, OCH_2), 4.42 (s, 2H, NCH_2CO), 7.15–7.98 (m, 9H, Ar–H), 11.98 (s, 1H, NHCO); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 14.1 (CH_3), 20.6 (CH_3), 54.6 (NCH_2), 61.5 (OCH_2), 110.6 (CN), 115.1 ($2CH_{Ar}$), 116.2 (C=N), 127.3 ($2CH_{Ar}$), 128.4 (C_{Ar}), 128.9 ($2CH_{Ar}$), 132.3 (CH_{Ar}), 133.3 ($2CH_{Ar}$), 133.8 (C_{Ar}), 146.0 (Oxadiazole-C_2), 147.3 (Oxadiazole-C_5), 151.2 (C_{Ar}), 164.5 (C = O), 169.8 (C=O). MS (EI, 70 eV) *m/z* (%): 432 (M^+, 5.2), 407 (11.1), 393 (10.3), 390 (4.1), 373 (1.2), 346 (16.3), 327 (0.3), 318 (0.3), 300 (0.5), 282 (0.5), 272 (0.2), 265 (1.3), 242 (6.2), 225 (0.6), 217 (1.2), 200 (0.3), 105 (100.0), 91 (11.5), 77 (36.1), 65 (4.1). Anal. Calcd. for $C_{22}H_{20}N_6O_4$ (432.43): C 61.10, H 4.66, N 19.43 %. Found: C 61.14, H 4.72, N 19.48 %.

Synthesis of ethyl 4-amino-3-(5-benzoylamino-1,3,4-oxadiazol-2-yl)-1-p-tolyl-1H-pyrazole-5-carboxylate (**4**) A mixture of compound **3** (0.43 g, 0.001 mol) in ethanolic sodium ethoxide solution [prepared by dissolving (0.2 g, 0.001 mol) of sodium metal in (25 mL) absolute ethanol) was refluxed for 6 h. The reaction mixture was allowed to cool down and then poured onto 50 mL ice-cold water containing few drops of dilute HCl. The resulting product was collected by filtration, dried and recrystallized from ethanol to give compound **4**.

Orange powder; Yield 67 %; m.p. 119–120 °C; IR (KBr): ν/cm^{-1} = 3345-3285 (NH_2), 3197 (NH), 1739 (CO, ester), 1703 (CO, amidic), 1600 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 1.26 (*t*, J = 7.5 Hz, 3H, CH_3), 2.32 (*s*, 3H, CH_3), 4.35 (*q*, J = 7.5 Hz, 2H, OCH_2), 6.35 (*s*, 2H, NH_2), 7.16–8.12 (*m*, 9H, Ar–H), 12.06 (s, 1H, NHCO); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 14.2 (CH_3), 20.7 (CH_3), 59.8 (OCH_2), 118.6 (Pyrazole-C_5), 124.9 ($2CH_{Ar}$), 127.8 ($2CH_{Ar}$), 128.9 ($2CH_{Ar}$), 131.2 ($2CH_{Ar}$), 132.4 (CH_{Ar}), 132.6 (Pyrazole-C_4), 133.0 (Pyrazole-C_3), 133.6 (C_{Ar}), 136.7 (C_{Ar}), 140.4 (C_{Ar}), 145.7 (Oxadiazole-C_2), 147.7 (Oxadiazole-C_5), 160.4 (C=O), 164.8 (C=O). MS (EI, 70 eV) *m/z* (%): 432 (M^+, 6.2), 407 (9.1), 393 (6.3), 390 (7.1), 373 (11.2), 346 (16.3), 327 (10.3), 318 (8.3), 300 (4.5), 282 (8.5), 272 (10.2), 265 (10.3), 242 (20.2), 225 (9.6), 217 (1.2), 200 (0.3), 105 (100.0), 91 (11.5), 77 (36.1), 65 (4.1). Anal. Calcd. for $C_{22}H_{20}N_6O_4$ (432.43): C 61.10, H 4.66, N 19.43 %. Found: C 61.12, H 4.70, N 19.38 %.

Synthesis of N-(5-(2-amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**5**) A mixture of compound **1** (0.23 g, 0.001 mol), cyclohexanone (0.10 g, 0.001 mol) and elemental sulfur (0.10 g, 0.003 mol) in ethanol (20 mL) and DMF (5 mL) containing morpholine (0.09 g, 0.001 mol) was heated on water bath at 50 °C for 6 h. After the reaction mixture was cooled, the solid product obtained was filtered off, dried well and recrystallized from ethanol to give compound **5**.

Green crystals; Yield 81 %; m.p. 179–180 °C; IR (KBr): ν/cm^{-1} = 3446–3388 (NH_2), 3195(NH), 1675(CO), 1600(C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 1.35–2.233 (*m*, 8H, Cyclohexane), 6.22 (*s*, 2H, NH_2), 7.24–7.98 (*m*, 5H, Ar–H), 12.01 (*s*, 1H, NHCO); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 21.1, 23.8, 26.3, 27.1 ($4CH_2$), 80.5 (Thiophene-C_3), 121.3 (Thiophene-C_4), 127.6 ($2CH_{Ar}$), 128.3 ($2CH_{Ar}$), 130.1 (Thiophene-C_5), 131.0 (C_{Ar}), 132.5 (CH_{Ar}), 143.9 (Oxadiazole-C_2), 154.5 (Oxadiazole-C_5), 162.9 (Thiophene-C_2), 165.0 (C=O). MS (EI, 70 eV) *m/z* (%): 342 (M^++2, 2.1), 340 (11.2), 339 (4.1), 327 (3.1), 313 (5.2), 299 (10.1), 285 (11.2), 278 (20.2), 250 (40.3), 236 (30.8), 235 (20.1), 208 (50.3), 180 (10.3), 160 (12.3), 152 (16.8), 128 (2.7), 123 (1.0), 111 (1.7), 96 (11.5), 83 (2.9), 64 (100). Anal. Calcd. for $C_{17}H_{16}N_4O_2S$ (340.4): C 59.98, H 4.74, N 16.46 %. Found: C 59.93, H 4.76, N 16.49 %.

Synthesis of N-(5-(1-cyano-4-(dimethylamino)-2-phenylbuta-1,3-dienyl)-1,3,4-oxadiazol-2-yl)benzamide (**6**) A mixture of compound **1** (0.46 g, 0.002 mol) and 3-(dimethylamino)-1-phenylprop-2-en-1-one (0.35 g, 0.002 mol) in DMF (10 mL) and TEA (0.2 mL) was refluxed for 6 h. The reaction mixture was left to cool and then poured onto ice-cold water (25 mL). The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound **6**.

Green crystals; Yield 68 %; m.p. 210–211 °C; IR (KBr): ν/cm^{-1} = 3222 (NH), 2213 (CN), 1678 (CO), 1625 (C=C). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 3.21 (*s*, 3H, CH_3), 3.34 (*s*, 3H, CH_3), 6.14 (*d*, *J* = 12.6 Hz, 1H, CH=), 6.36 (*d*, *J* = 12.6 Hz, 1H, CH =), 7.14–8.05 (*m*, 10H, Ar–H), 11.98 (*s*, 1H, NHCO); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 41.2 ($N(CH_3)_2$), 93.8 (C=), 98.3 (C=), 117.4 (CN), 127.3 ($2CH_{Ar}$), 128.0 ($2CH_{Ar}$), 129.2 ($2CH_{Ar}$), 130.0 ($2CH_{Ar}$), 130.6 (CH_{Ar}), 131.4 (CH_{Ar}), 132.8 (C_{Ar}), 135.6 (C_{Ar}), 143.9 (Oxadiazole-C_2), 145.2 (Oxadiazole-C_5), 149.6 (C=N), 154.4 (C=), 164.9 (C=O). MS (EI, 70 eV) *m/z* (%): 386 (M^++1, 1.5), 385 (9.7), 369 (2.3), 356 (1.8), 345 (4.1), 329 (3.2), 321 (2.2), 316 (10.7), 315 (1.7), 298 (2.3), 291 (1.5), 280 (1.4) 279 (2.6), 264 (3.9), 238 (3.0), 240 (4.7), 224 (2.1), 213 (1.1), 212 (8.5), 105 (100), 77 (67.8). Anal. Calcd. for $C_{22}H_{19}N_5O_2$ (385.42): C 68.56, H 4.97, N 18.17 %. Found: C 68.60, H 5.02, N 18.24 %.

General procedure for the Synthesis of pyrano[2,3-c]pyrazole **11** *and chromene* **12** *derivatives* To a solution of compound **1** (0.23 g, 0.001 mol) and *p*-anisaldehyde (0.12 mL, 0.001 mol), in ethanol (25 mL) containing a few drops of piperidine, either 1-phenyl-3-methyl-2-pyrazolin-5-one (0.17 g, 0.001 mol) or 5,5-dimethyl-1,3-cyclohexanedione (0.14 g, 0.001 mol) was added. The reaction mixture, in each case, was heated under reflux for 24 h. The solid products formed upon pouring onto an ice-cold-water mixture (25 mL) containing few drops of hydrochloric acid

was collected by filtration, dried, and recrystallized from DMF to afford compounds **11** and **12**, respectively.

N-(5-(6-Amino-4-(4-methoxyphenyl)-3-methyl-1-phenyl-1,4-di-hydropyrano[2,3-c] pyrazol-5-yl)-1,3,4-oxadiazol-2-yl)benzamide (**11**) Pale yellow powder; Yield 83 %; m.p. 230–231 °C; IR (KBr): ν/cm^{-1} = 3451–3418 (NH_2), 3190 (NH), 1666 (C=O), 1610 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 2.32 (*s*, 3H, CH_3), 3.83 (*s*, 3H, OCH_3), 4.84 (*s*, 1H, pyran-H-4), 7.12–8.12 (*m*, 16H, Ar–H, NH_2), 12.31 (*s*, 1H, NH amidic); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 13.4 (CH_3), 33.4 (Pyran-C_4), 55.2 (OCH_3), 91.9 (Pyran-C_3), 98.2 (Pyrazole-C_{4a}), 117.1 ($2CH_{Ar}$), 119.5 ($2CH_{Ar}$), 124.8 (CH_{Ar}), 127.5 ($2CH_{Ar}$), 127.8 ($2CH_{Ar}$), 128.8 ($2CH_{Ar}$), 129.1 ($2CH_{Ar}$), 129.5 (CH_{Ar}), 131.1 (C_{Ar}), 132.2 (CH_{Ar}), 137.0 (C_{Ar}), 138.0 (C_{Ar}), 142.1 (Pyrazole-C_2), 142.3 (Pyrazole-C_3), 145.6 (Oxadiazole-C_2), 149.3 (Oxadiazole-C_5), 161.4 (C_{Ar}), 164.8 (C=O). MS (EI, 70 eV) *m/z* (%): 520 (M^+, 12.5), 445 (10.1), 428 (5.2), 415 (8.2), 400 (10.2), 384 (6.2), 370 (11.6), 361 (9.1), 346 (14.8), 345 (20.2), 331 (0.2), 318 (4.1), 292 (8.1), 279 (6.3), 264 (4.1), 241 (8.3), 226 (10.2), 211 (11.1), 200 (20.6), 186 (4.4), 171 (10.4), 158 (2.9), 143 (2.3), 127 (1.9), 115 (3.8), 105 (100), 88 (1.8), 77 (43.0), 63 (1.9). Anal. Calcd. for $C_{29}H_{24}N_6O_4$ (520.19): C 66.91, H 4.65, N 16.14 %. Found: C 66.82, H 4.61, N 16.11 %.

N-(5-(2-Amino-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**12**) Pale yellow powder; Yield 79 %; m.p. 120–121 °C; IR (KBr): ν/cm^{-1} = 3380–3324 (NH_2), 2999 (NH), 1668 (C=O), 1645 (C=O), 1599 (C=N).^{1}H–NMR (DMSO-d_6): δ_{ppm} = 1.29 (*s*, 6H, $2CH_3$), 1.69 (*s*, 2H, CH_2), 2.45 (*s*, 2H, CH_2), 3.79 (*s*, 3H, OCH_3), 3.88 (*s*, 1H, CH), 6.10 (*s*, 2H, NH_2), 6.75–8.27 (*m*, 9H, Ar–H), 12.78 (*s*, 1H, NH); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 26.8 (CH_3), 28.2 (CH_3), 31.7 (C_q), 39.6 (CH_2), 40.4 (CH), 44.2 (CH_2), 55.2 (OCH_3), 91.3 (Chromene-C_3), 111.5 (C=), 114.7 ($2CH_{Ar}$), 127.0 ($2CH_{Ar}$), 127.8 ($2CH_{Ar}$), 128.9 ($2CH_{Ar}$), 130.2 (C_{Ar}), 132.4 (CH_{Ar}), 138.6 (C_{Ar}), 145.2 (Oxadiazole-C_2), 154.2 (Chromene-C_2), 155.3 (Oxadiazole-C_5), 157.2 (C_{Ar}-O), 160.5 (O–C=), 164.8 (C=O), 196.2 (C=O). MS (EI, 70 eV) *m/z* (%): 486 (M^+,8.0), 457 (5.4), 456 (0.7), 455 (8.0), 441 (11.7), 432 (6.5), 415 (100), 400 (7.7), 379 (9.4), 370 (18.2), 361 (13.6), 346 (32.8), 324 (9.3), 316 (8.9), 295 (41.6), 281 (24.4), 273 (14.1), 253 (8.9), 250 (14.4), 236 (8.3), 211 (13.2), 197 (13.6), 178 (6.3), 164 (11.3), 149 (8.8), 135 (6.2), 120 (4.1), 111 (19.3). Anal. Calcd. for $C_{27}H_{26}N_4O_5$ (486.52): C 66.65, H 5.39, N 11.52 %. Found: C 66.69, H 5.43, N 11.58 %.

*Synthesis of N-(5-(5,7-dimethyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (***15**) A mixture of compound **13** (0.36 g, 0.001 mol) and acetylacetone (0.13 g, 0.001 mol) in acetic acid (20 mL) was refluxed for 3 h. The reaction mixture was left to cool and then poured into ice-cold water. The resulting solid product was collected and recrystallized from DMF to give compound **15**.

Grey powder; Yield 76 %; m.p.214–215 °C; IR (KBr): ν/cm^{-1} = 3332, 3265 (2NH), 1664 (amidic CO), 1596 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 2.61 (*s*, 3H, CH_3), 2.75 (*s*, 3H, CH_3), 6.95 (*s*, 1H, Pyrimidine-H_5), 7.05–7.94 (m, 10H, Ar–H), 10.65 (*s*, 1H, NHPh), 11.36 (s, 1H, NHCOPh); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 15.7 (CH_3), 19.4 (CH_3), 98.1 (Pyrazole-C_4), 107.9 (Pyrimidine-C_5), 118.5 ($2CH_{Ar}$),

119.5 (CH_{Ar}), 127.9 ($2CH_{Ar}$), 128.8 ($2CH_{Ar}$), 129.1 ($2CH_{Ar}$), 130.1 (Pyrazole-C_3), 131.2 (C_{Ar}), 132.4 (CH_{Ar}), 144.3 (Oxadiazole-C_2), 145.7 (C_{Ar}), 149.5 (Pyrimidine-C_4), 151.2 (Oxadiazole-C_5), 156.3 (Pyrimidine-C_2), 163.6 (Pyrimidine-C_6), 164.5 (C=O). MS (EI, 70 eV) *m/z* (%): 425 (M^+, 6.1), 415 (3.2), 392 (5.2), 381 (1.2), 369 (8.1), 352 (8.4), 338 (1.1), 322 (12.4), 321 (10.3), 296 (1.2), 281 (4.5), 266 (20.3), 265 (100), 237 (1.8), 221 (1.5), 196 (1.3), 186 (1.9), 174 (2.7), 161 (1.1), 146 (1.9), 137 (16.02), 118 (1.9), 103 (6.6), 92 (2.6), 77 (8.2), 65 (5.3). Anal. Calcd. for $C_{23}H_{19}N_7O_2$ (425.44): C 64.93, H 4.50, N 23.05 %. Found: C 65.02, H 4.54, N 23.13 %.

Synthesis of N-(5-(7-methyl-5-oxo-2-(phenylamino)-4,5-dihydro-pyrazolo[1,5-a]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**17**) A mixture of compound **13** (0.36 g, 0.001 mol) and ethyl acetoacetate (0.13 g, 0.001 mol) or acetoacetanilide (0.177 g, 0.001 mol) in glacial acetic acid (20 mL) was refluxed for 3 h. The reaction mixture was left to cool down and then poured onto ice-cold water (25 mL). The resulting solid was collected by filtration, dried, and recrystallized from DMF to give compound **17**.

Grey powder; Yield 71 %; m.p. 229–230 °C; IR (KBr): ν/cm^{-1} = 3460 (OH), 3340, 3215 (2NH), 1661 (amidic CO), 1600 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 2.36 (*s*, 3H, CH_3), 6.90 (*s*, 1H, pyrimidine-H_5), 7.24–7.95 (*m*, 10H, Ar–H), 10.19 (*s*, br, 1H, NH), 11.47 (*s*, 1H, NHCOPh), 12.63 (*s*, 1H, OH); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 19.6 (CH_3), 89.9 (Pyrimidine-C_5), 98.4 (C=), 118.2 ($2CH_{Ar}$), 119.8 (CH_{Ar}), 127.0 ($2CH_{Ar}$), 128.5 ($2CH_{Ar}$), 129.4 ($2CH_{Ar}$), 131.0 (C_{Ar}), 132.4 (CH_{Ar}), 133.5 (C=N), 144.0 (Oxadiazole-C_2), 145.7 (C_{Ar}), 148.5 (C=), 151.2 (Oxadiazole-C_5), 164.9 (C=O), 167.1 (C=N), 174.5 (Pyrimidine-C_4). MS (EI, 70 eV) *m/z* (%) = 427 (M^+, 8.3), 419 (1.6), 393 (1.4), 388 (1.9), 360 (8.1), 346 (19.8), 323 (11.5), 309 (6.5), 259 (3.1), 283 (32.0), 226 (32.8), 256 (37.4), 240 (71.8), 237 (15.7), 215 (35.8), 209 (13.4), 189 (12.8), 184 (100.0), 160 (29.8), 146 (16.2), 129 (5.4), 118 (30.6), 103 (93.6), 91 (21.9), 77 (86.6), 68 (12.5). Anal. Calcd. for $C_{22}H_{17}N_7O_3$ (427.42): C 61.82, H 4.01, N 22.94 %. Found: C 61.88, H 4.07, N 23.02 %.

Synthesis of N-(5-(3-(benzylideneamino)-5-(phenylamino)-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)benzamide (**20**) Equimolar amounts of compound **13** (0.36 g, 0.001 mol) and benzaldehyde (0.11 g, 0.001 mol) in ethanol (20 mL) containing a catalytic amount of piperidine was refluxed for 3 h. The reaction mixture was left to cool and then poured onto ice-cold water (15 mL). The obtained solid product was collected by filtration, dried, and recrystallized from DMF to give compound **20**.

Yellow powder; Yield 62 %; m.p. 299–300 °C; IR (KBr): ν/cm^{-1} = 3440, 3315, 3181 (3NH), 1650 (C=O), 1600 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 6.82 (*s*, 1H, CH=), 7.17–7.92 (*m*, 16H, Ar–H, NH), 11.85 (*s*, br, 1H, NH). MS (EI, 70 eV) *m/z* (%): 449 (M^+, 12.1), 434 (7.9), 432 (3.2), 408 (1.0), 403 (2.2), 389 (1.6), 369 (5.2), 359 (0.2), 344 (0.15), 343 (0.2), 329 (0.3), 313 (0.9), 304 (41.6), 289 (100), 266 (2.0), 265 (10.8), 262 (1.8), 261 (2.1), 238 (12.7), 231 (2.7), 211 (5.5), 200 (17.1), 185 (7.6), 174 (16.6), 171 (2.4), 170 (1.2), 156 (5.9), 146 (6.1), 128 (7.0), 118 (8.7),

104 (33.6), 91 (17.2), 84 (2.9), 77 (50.9). Anal. Calcd. for $C_{25}H_{19}N_7O_2$ (449.46): C 66.81, H 4.26, N 21.81 %. Found: C 66.78, H 4.24, N 21.72 %.

Synthesis of N-(5-(7-amino-6-cyano-5-phenyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-1,3,4-oxadiazol-2-yl)benzamide (**19**) *Method A*: Equimolar amounts of compound **13** (0.36 g, 0.001 mol) and α-cyanocinnamonitrile (0.15 g, 0.001 mol) in ethanol (20 mL) containing a catalytic amount of piperidine was refluxed for 4 h. The reaction mixture was left to cool down and then poured onto ice-cold water (15 mL). The solid obtained was collected by filtration, dried, and recrystallized from DMF to give compound **19**.

Method B: Equimolar amounts of Schiff base **20** (0.45 g, 0.001 mol) and malononitrile (0.08 g, 0.001 mol) in ethanol (10 mL) containing a catalytic amount of piperidine was refluxed for 4 h. The reaction mixture was left to cool and then poured into ice-cold water (10 mL). The obtained product was collected by filtration and recrystallized from DMF to give compound **19**.

Brown powder; Yield 73 %; m.p. 229–230 °C; IR (KBr): ν/cm^{-1} = 3435–3395 (NH_2), 3292, 3197 (2NH), 2191 (CN), 1653 (C=O), 1600 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 5.91 (*s*, 2H, NH_2), 7.32–7.90 (*m*, 15H, Ar–H), 9.38 (*s*, 1H, NH), 11.58 (*s*, 1H, NH); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 74.2 (Pyrimidine-C_5), 97.8 (C=), 114.8 (CN), 118.2 ($2CH_{Ar}$), 119.1 (CH_{Ar}), 121.4 ($2CH_{Ar}$), 127.5 ($2CH_{Ar}$), 128.7 ($2CH_{Ar}$), 129.0 ($2CH_{Ar}$), 129.8 (CH_{Ar}), 130.2 ($2CH_{Ar}$), 131.4 (C_{Ar}), 132.2 (CH_{Ar}), 133.1 (C=N), 137.8 (C_{Ar}), 144.0 (Oxadiazole-C_2), 145.0 (C=), 145.7 (C_{Ar}), 150.8 (Oxadiazole-C_5), 155.4 (Pyrimidine-C_6), 164.4 (C=O), 169.8 (Pyrimidine-C_4). MS (EI, 70 eV) *m/z* (%): 513 (M^+, 9.5), 455 (11.4), 442 (1.5), 431 (8.6), 419 (9.6), 393 (10.7), 382 (12.2), 372 (4.8), 356 (11.8), 336 (13.2), 303 (25.0), 277 (14.4), 261 (17.6), 235 (23.2), 219 (36.7), 208 (34.8), 185 (18.98), 178 (28.0), 166 (32.0), 141 (39.7), 136 (23.9), 118 (100.0), 109 (27.5), 89 (35.8), 83 (34.4), 65 (88.3). Anal. Calcd. for $C_{28}H_{19}N_9O_2$ (513.51): C 65.49, H 3.73, N 24.55 %. Found: C 65.53, H 3.76, N 24.62 %.

*General procedure for the Synthesis of imidazol[1,2-b]pyrazole derivatives 2 and 2***24** To a solution of compound **13** (0.36 g, 0.001 mol) in dioxane (25 mL) containing two drops of triethylamine, either chloroacetyl chloride (0.08 ml, 0.001 mol), or phenacyl bromide (0.2 g, 0.001 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h. The solid products formed upon pouring onto an ice-cold water (15 mL) containing few drops of hydrochloric acid was collected by filtration, dried, and recrystallized from a mixture of ethanol/DMF (3:1) to afford compounds **22** and **24**, respectively.

N-(5-(3-Oxo-6-(phenylamino)-2,3-dihydro-1H-imidazo[1,2-b]pyrazol-7-yl)-1,3,4-oxadiazol-2-yl)benzamide (**22**) Yellow powder; Yield 79 %; m.p. 246–247 °C; IR (KBr): ν/cm^{-1} = 3326, 3198, 3154 (3NH), 1690 (CO, cyclic), 1663 (C=O, amidic), 1584 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 4.05 (*d*, J = 6.6 Hz, 2H, CH_2), 7.32–7.93 (*m*, 10H, Ar–H), 10.25 (br, *s*, 1H, NH), 11.50 (br, *s*, 1H, NH), 12.36 (br, *s*, 1H, NH); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 47.7 (NCH_2), 83.1 (Pyrazole-C_4), 118.5 ($2CH_{Ar}$), 119.5 (CH_{Ar}), 127.8 ($2CH_{Ar}$), 128.9 ($2CH_{Ar}$), 129.7 ($2CH_{Ar}$), 131.1 (C_{Ar}), 132.6 (CH_{Ar}), 140.6 (C_{Ar}), 144.3 (Oxadiazole-C_2), 145.3 (Pyrazole-C_3), 147.0

(Oxadiazole-C_5), 153.8 (Imidazole-C_2), 164.1 (C=O), 165.9 (C=O). MS (EI, 70 eV) *m/z* (%): 401 (M^+, 4.3), 344 (1.5), 285 (1.7), 253 (100), 225 (2.2), 214 (4.3), 181 (12.6), 117 (8.3), 105 (6.5), 99 (14.2), 91 (11.9), 84 (18.9), 73 (33.0), 69 (15.5). Anal. Calcd. for $C_{20}H_{15}N_7O_3$ (401.38): C 59.85, H 3.77, N 24.43 %. Found: C 59.91, H 3.83, N 24.47 %.

N-(5-(3-Phenyl-6-(phenylamino)-1H-imidazo[1,2-b]pyrazol-7-yl)-1,3,4-oxadiazol-2-yl)benzamide (**24**) Orange powder; Yield 82 %; m.p. 230–231 °C; IR (KBr): ν/cm^{-1} = 3330, 3200, 3160 (3NH), 1661 (CO, amidic), 1587 (C=N). ^{1}H–NMR (DMSO-d_6): δ_{ppm} = 6.24 (*d*, *J* = 6.6 Hz, 1H, CH=), 7.17–7.87 (m, 15H, Ar–H), 10.25 (*s*, 1H, NH), 11.37 (*s*, 1H, NH), 12.66 (*s*, 1H, NH); ^{13}C–NMR (DMSO-d_6): δ_{ppm} = 78.5 (Pyrazole-C_4), 118.3 (2CH_{Ar}), 119.1 (CH_{Ar}), 121.4 (2CH_{Ar}), 123.3 (Imidazole-C_4), 126.4 (CH_{Ar}), 127.6 (2CH_{Ar}), 128.5 (2CH_{Ar}), 129.0 (2CH_{Ar}), 130.2 (2CH_{Ar}), 130.9 (Imidazole-C_5), 131.3 (C_{Ar}), 132.4 (CH_{Ar}), 134.3 (C_{Ar}), 138.4 (Imidazole-C_2), 138.9 (Pyrazole-C_3), 143.2 (C_{Ar}), 144.5 (Oxadiazole-C_2), 149.6 (Oxadiazole-C_5), 164.8 (C=O). MS (EI, 70 eV) *m/z* (%): 461 (M^+, 5.5), 425 (1.3), 382 (1.6), 353 (1.0), 340 (12.2), 295 (1.8), 268 (1.1), 248 (8.4), 227 (24.4), 188 (1.5), 158 (1.5), 122 (1.8), 115 (5.5), 95 (100.0), 88 (6.8), 76 (36.5), 65 (1.9). Anal. Calcd. for $C_{26}H_{19}N_7O_2$ (461.47): C 67.67, H 4.15,N 21.25 %. Found: C 67.72, H 4.22, N 21.24 %.

Antioxidant screening using ABTS method [40]

The antioxidant activity was evaluated from the bleaching of ABTS derived radical cations. The radical cations derived from ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 µL) with MnO_2 (3 mL, 25 mg/mL) in 5 mL aqueous phosphate buffer (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 equation:

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

Ascorbic acid (20 µL, 2 mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS and using MeOH/phosphate buffer (1:1) instead of a sample. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) instead of test compounds. The results of antioxidant activity were depicted in Table 1.

Bleomycin-dependent DNA damage

The assay was done with minor modifications following a method described earlier by Aeschbach et al. [42] and Chan and Tang [43]. The reaction mixture (0.5 mL) contained DNA (0.5 g/mL), Bleomycin sulfate (0.05 mg/mL), and $MgCl_2$ (5 mM),

$FeCl_3$ (50 mM) and the samples were dissolved in DMSO to be tested at a concentration (20 μg of 1 mg/mL). L-Ascorbic acid was used as a positive control. The reaction was terminated by addition of EDTA (0.05 mL, 0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL, 1 %, w/v) and HCl (0.5 mL, 25 %, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm (Table 2).

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