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ORIGINAL PAPER

Synthesis and evaluation of new phenolic derivatives as antimicrobial and antioxidant agents

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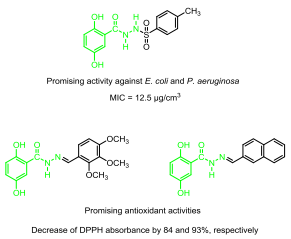
Abstract New phenolic derivatives bearing hydrazine and 1,3,4-oxadiazole moieties were synthesized and evaluated for their in vitro antimicrobial and antioxidant activities. Most of the compounds revealed pronounced activity against *Pseudomonas aeruginosa* as well as promising antioxidant activities. N^1-(2,5-Dihydroxybenzoyl)-N^2-(4-methylphenylsulfonyl)hydrazine displayed promising activity against *Escherichia coli* and *P. aeruginosa*. N^1-(2,5-Dihydroxybenzoyl)-N^2-(2-naphthalenylmethylene)hydrazine was almost equipotent to the standard antioxidant vitamin C having scavenging activities of 84 and 93%, respectively. In vitro cytotoxicity study revealed that N^1-(2,5-dihydroxybenzoyl)-N^2-(2,3,4-trimethoxyphenylmethylene)hydrazine, N^1-(2,5-dihydroxybenzoyl)-N^2-(3,4,5-trimethoxyphenylmethylene)hydrazine, and N^1-(2,5-dihydroxybenzoyl)-N^2-(4-methylphenylsulfonyl)hydrazine are more safe than reference 5-fluorouracil. In silico drug relevant properties proposed that all compounds have high to moderate drug-likeness scores. Accordingly, these derivatives can be potential leads for development of potent antimicrobial and antioxidant agents.

Graphical abstract



Keywords Phenolic derivatives · Hydrazine · 1,3,4-Oxadiazole · Synthesis · Antimicrobial activity · Antioxidant activity

Introduction

The incidence of fungal and bacterial infections has increased dramatically in the past years. The widespread use of antifungal and antibacterial drugs together with resistance of fungal and bacterial infections to antimicrobial agents has led to serious health hazards. This has initiated the discovery and modification of new antifungal and antibacterial drugs [1]. Moreover, oxidative stress plays a central role in the development of human diseases [2]. Free radicals and reactive oxygen species (ROS) are constantly formed the human body and are involved in the growth, differentiation, progression, and death of the cell. However, accumulation of high amounts of ROS causes a number of human diseases, including arthritis, cancer, diabetes, atherosclerosis, ischemia, failures in immunity

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and endocrine functions. Antioxidants act as safeguard against the accumulation of ROS and their elimination from the system [2–4].

Phenolic derivatives are of current interest due to their wide-spectrum bioactivity and chemotherapeutic value. Furthermore, naturally occurring phenolic compounds exhibit antimicrobial [5–7] and antioxidant [5, 7–12] activities owing to their role as free radical scavengers [13]. Accordingly, phenolic nucleus is considered an important scaffold for the synthesis of efficient antioxidant [14–17] and broad spectrum antimicrobial agents [14–16]. The effects of incorporating various substituents to phenolic derivatives for enhancement of their antioxidant activities was studied extensively [18, 19]. (*E*)-1-(2,5-Dihydroxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one exhibited very good free radical scavenging property in DPPH assay [20]. In addition, molecules possessing more phenolic hydroxyl groups proved to have stronger DPPH scavenging ability [21].

Besides, various heterocycles have proven to be important scaffolds for the design of new biologically active compounds [22, 23], among them, the five-membered 1,3,4-oxadiazoles which are considered as an important class of heterocyclic compounds possessing antimicrobial [24–26] and antioxidant activities [24, 25]. 1,3,4-Oxadiazoles are good bioisosteres of amides and esters, which may enhance interactions with the receptors through hydrogen bonding, thus may contribute in potentiating the pharmacological activity [27]. Moreover, hydrazines exhibit good antimicrobial [28–32] and antioxidant [32–35] activities. Incorporating hydrazine moiety to phenolic compounds increased the antioxidant potency [21, 33]. Phenolic phloretin isonicotinyl hydrazone exhibited higher DPPH scavenging ability and inhibition of lipid peroxidation than phloretin [21].

Accordingly, as a continuation of our previous studies which involved the discovery of new phenolic derivatives exhibiting antimicrobial activity [36, 37], we present in this study the antimicrobial and antioxidant evaluation of newly synthesized phenolic derivatives linked to hydrazines and various 1,3,4-oxadiazoles directly attached to the phenolic nucleus hoping to impart some synergism to the prepared compounds. Computational study for the determination of Lipinski parameters as well as the prediction of drug-likeness and toxicity of the active compounds were performed.

Results and discussion

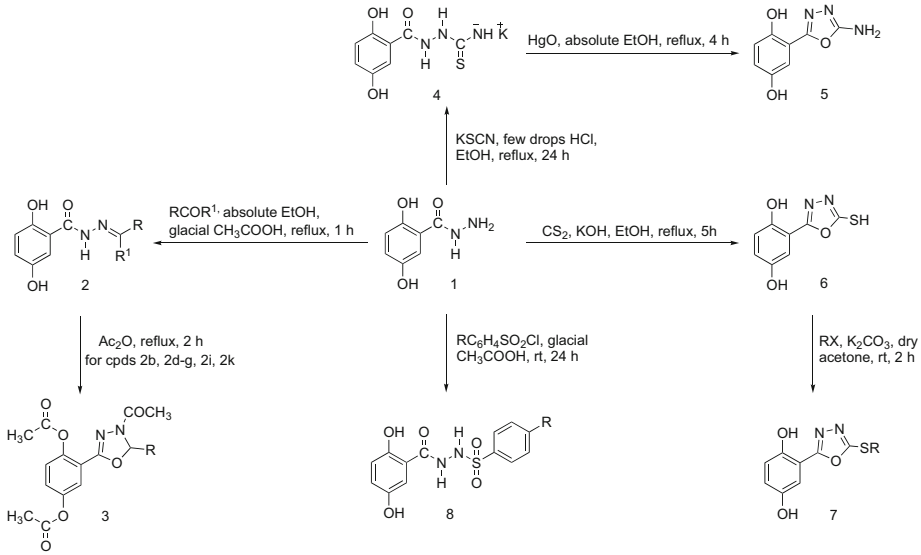
Chemistry

Synthesis of the target compounds was performed according to the reactions in outlined Scheme 1. 2,5-Dihydroxybenzohydrazide (**1**) was prepared by reacting methyl 2,5-dihydroxybenzoate with hydrazine hydrate according to the reported reaction conditions [38]. Reacting compound **1** with various aldehydes and ketones yielded the corresponding hydrazines **2a–2q** in yields ranging from 51 to 94%. Cyclization of some selected compounds **2a**, **2d–2g**, **2i**, and **2k** using acetic anhydride yielded the corresponding acetoxyoxadiazolylphenyl acetates **3a–3g** in yields ranging from 57 to 85%. I.R. spectra of compounds **3a–3g** showed the characteristic ester and amide C=O absorption bands as well as the stretching absorption bands due to the C=N and C–O–C functions. ^{1}H NMR spectra lacked the singlet assigned for the methine (CH=N) proton and the deuterium-exchangeable singlets assigned for the NH and OH protons, whereas they showed the appearance of singlets assigned for the $COCH_3$ and $OCOCH_3$ protons and the C5-oxadiazolyl proton. Reacting compound **1** with potassium thiocyanate afforded compound **4** which was subjected to cyclodesulfurization using yellow mercuric oxide to yield 52% of 5-amino-1,3,4-oxadiazole **5**. IR spectrum of compound **5** revealed the absorption band of the NH_2 group as well as stretching absorption bands for OH, C=N, and C–O–C functions at their expected positions. ^{1}H NMR spectrum revealed the appearance of three deuterium-exchangeable singlets assigned for the NH_2 and OH protons besides other protons at their expected chemical shifts. Compound **1** was also reacted with carbon disulfide and potassium hydroxide to afford the target sulfanyloxadiazole **6**. IR spectrum of this compound revealed the absorption band of the SH group as well as stretching absorption bands for OH, C=N, and C–O–C functions at their expected positions. In addition, its ^{1}H NMR spectrum showed the appearance of three deuterium-exchangeable singlets assigned for the OH and SH protons besides aromatic protons at their expected chemical shifts. Compound **6** was alkylated using the appropriate alkyl or aralkyl halide to yield the corresponding substituted sulfanyloxadiazoles **7a–7c** in 73 and 78% yields. Compound **1** was then reacted with arylsulfonylchlorides to yield the target arylsulfonylhydrazines **8a**, **8b** in 44 and 67% yields, respectively. Structures of the newly synthesized compounds **2a–2q**, **3a–3g**, **5**, **6**, **7a–7c**, and **8a**, **8b** were confirmed by studying their spectral and analytical data.

Antioxidant activity: DPPH radical scavenging assay

Free radical scavenging activity of seventeen of the newly synthesized compounds **2c–2e**, **2h–2j**, **2m**, **2p**, **2q**, **3a**, **3b**, **3f**, **7a**, **7c**, **5**, **8a**, and **8b** was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test [39] using four different concentrations (0.25, 0.5, 0.75, and 1 mg/cm^3). The odd electron in DPPH free radical

Scheme 1



Comp.	R	R^1	Comp.	R	R^1
2a	4-imidazolyl	H	**2p**	2-oxoindolin-3-ylidene	
2b	4-hydroxyphenyl	H	**2q**	5-iodo-2-oxoindolin-3-ylidene	
2c	2,3,4-trihydroxyphenyl	H	**3a**	4-acetoxynaphthalene	-
2d	4-hydroxy-3-methoxy phenyl	H	**3b**	4-acetoxy-3-methoxy phenyl	-
2e	3-hydroxy-4-methoxy phenyl	H	**3c**	3-acetoxy-4-methoxy phenyl	-
2f	4-hydroxy-3-ethoxy phenyl	H	**3d**	4-acetoxy-3-ethoxyphenyl	-
2g	3,4-dimethoxyphenyl	H	**3e**	3,4-dimethoxyphenyl	-
2h	2,3,4-trimethoxyphenyl	H	**3f**	3,4,5-trimethoxyphenyl	-
2i	3,4,5-trimethoxyphenyl	H	**3g**	2-acetoxy-1-naphthalenyl	-
2j	2-naphthalenyl	H	**7a**	methyl	-
2k	2-hydroxy-1-naphthalenyl	H	**7b**	ethyl	-
2l	5-bromo-3-indolyl	H	**7c**	benzyl	-
2m	2-pyridinyl	methyl	**8a**	H	-
2n	2-pyrazinyl	methyl	**8b**	methyl	-
2o	1-naphthalenyl	methyl			

produces strong absorption in the visible region at 517 nm producing a purple color. In the presence of an antioxidant, DPPH free radical is reduced to DPPH-H (2,2-diphenyl-1-picrylhydrazine) which gives a yellow color. This color change is stoichiometric so the extent of discoloration is proportional to the scavenging ability of the tested compound. The antioxidant potential of the tested compounds was calculated as the decrease of DPPH absorbance % mean ± SD in relation to control and vitamin C which was used as the reference standard antioxidant.

Most of the tested compounds showed moderate radical scavenging activities at low concentrations. However, there was an increase in scavenging activities proportional with increasing concentrations for most of the compounds. Results are recorded in Table 1 and Fig. 1 shows the graphical representation of the antioxidant activity of tested compounds. Efficacy is defined as the maximum effect that a drug can produce regardless of its dose. Efficacy of each of the tested compounds was calculated by dividing the decrease of DPPH absorbance % produced by 1 mg of the compound with that produced by 1 mg of vitamin C.

Besides their antioxidant activity, phenolic derivatives can act as prooxidants [11]. In the presence of oxygen, transition metals such as Cu and Fe catalyze the redox cycling of phenolics, leading to the formation of reactive oxygen species and other organic radicals that can damage

Table 1 DPPH radical scavenging activity of compounds **2c–2e**, **2h–2j**, **2m**, **2p**, **2q**, **3a**, **3b**, **3f**, **7a**, **7c**, **5**, **8a**, and **8b**

Comp.	Decrease of DPPH absorbance % mean ± SD ($n = 3$)				
	0.25 mg/cm^3	0.5 mg/cm^3	0.75 mg/cm^3	1 mg/cm^3	Efficacy[a]
2c	54.0 ± 2.1[b]	61.0 ± 4.2	64.0 ± 5.4	54.5 ± 4.8	0.58
2d	47.0 ± 1.5	46.0 ± 1.9	45.0 ± 4.1	49.0 ± 1.6	0.52
2e	43.0 ± 4.2	45.0 ± 1.3	45.0 ± 4.2	72.0 ± 3.8	0.77
2h	32.0 ± 3.1	32.0 ± 2.1	44.0 ± 3.4	84.0 ± 5.8	0.89
2i	74.0 ± 1.1	71.0 ± 1.1	70.0 ± 1.0	41.0 ± 0.8	0.44
2j	68.0 ± 0.9	84.0 ± 1.1	92.0 ± 1.2	93.0 ± 1.3	0.99
2m	−40.2 ± 2	25.0 ± 2.5	22.0 ± 2.1	23.0 ± 2.1	0.24
2p	56.0 ± 1.5	46.0 ± 1.5	46.0 ± 0.3	52.0 ± 1.8	0.55
2q	−16.0 ± 1.2	11.0 ± 1.1	0 ± 0	5.6 ± 0.5	0.06
3a	71.0 ± 5.2	65.0 ± 5.5	61.0 ± 4.2	43.0 ± 5.8	0.46
3b	29.0 ± 0.8	30.0 ± 0.7	36.0 ± 1.1	40.0 ± 1.2	0.43
3f	37.0 ± 4.0	48.0 ± 3.2	53.0 ± 4.1	63.0 ± 3.9	0.67
5	52.4 ± 4.2	53.0 ± 1.8	57.0 ± 7.1	64.0 ± 4.9	0.68
7a	31.0 ± 2.1	39.0 ± 1.5	48.0 ± 1.8	49.8 ± 4.7	0.53
7c	62.0 ± 3.5	54.0 ± 2.5	45.0 ± 4.1	44.4 ± 4.5	0.47
8a	6.0 ± 0.5	6.0 ± 0.5	73.0 ± 1.1	79.0 ± 1.2	0.84
8b	−31.0 ± 1.1	16.0 ± 0.3	21.0 ± 0.8	60.0 ± 1.2	0.64
Vitamin C[c]	72.0 ± 2.1	77.0 ± 3.5	98.0 ± 5.6	94.0 ± 1.9	

[a]1 mg of compound/1 mg of vitamin C

[b]Mean ± SD, $n = 3$

[c]Reference standard antioxidant

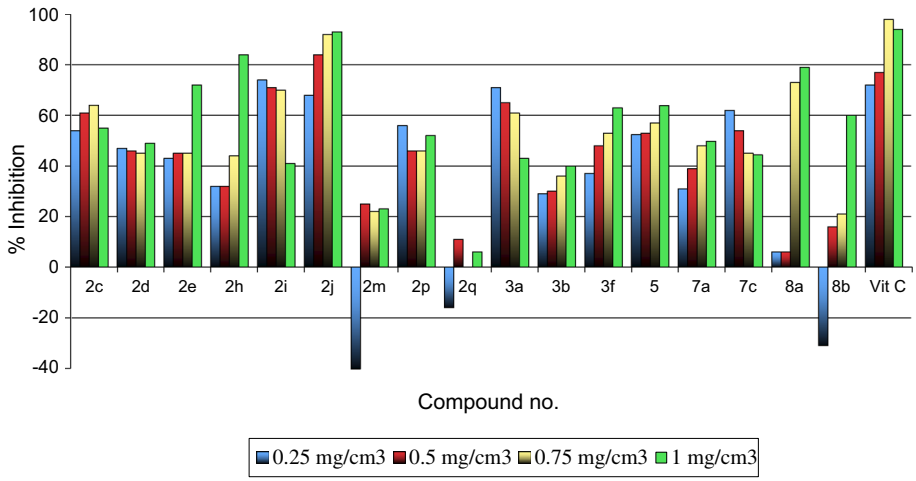


Fig. 1 DPPH scavenging activity of compounds **2c–2e**, **2h–2j**, **2m**, **2p**, **2q**, **3a**, **3b**, **3f**, **7a**, **7c**, **5**, **8a**, and **8b**

DNA, lipids, and other biological molecules [40]. Accordingly, antioxidants which are free radical scavengers, decrease the absorbance intensity, whereas prooxidants (which increase the concentration of free radicals) increase the absorbance intensity relative to the reference system [41, 42].

This proves the results obtained with compounds **2c**, **2d**, **2i**, **2m**, **2p**, **3a**, and **7c** which at certain concentrations did not show an increase in radical scavenging activities with increasing concentrations as they might act as prooxidants. Also some compounds **2m**, **2q**, and **8b** displayed negative % inhibition values which indicates that they might act as prooxidants that can cause initiation of lipid peroxidation.

Hydrazines **2h** and **2j** were found to be the most potent derivatives. At 1 mg/cm^3 concentration, they showed scavenging activities of 84 and 93%, respectively. They were almost equipotent to vitamin C, the reference standard antioxidant used, which displayed scavenging activity

of 94%. Compounds **2e**, **3f**, **5**, **8a**, and **8b** exhibited moderate antioxidant potencies. At 1 mg/cm^3 concentration, they revealed scavenging activities of 72, 63, 64, 79, and 60%, respectively. Compounds **2c**, **2d**, **2p**, **3a**, **3b**, **7a**, and **7c** showed mild scavenging activities having 55, 49, 52, 43, 40, 50, and 44% scavenging activities at 1 mg/cm^3 concentration level.

Antimicrobial screening

In vitro antimicrobial screening of the thirty-one newly prepared phenolic derivatives was performed against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *P. aeruginosa*), and the fungus *Candida albicans*.

Most of the compounds showed promising activity against *P. aeruginosa*. Methylphenylsulfonylhydrazine **8b** was four times more active than ampicillin against *P. aeruginosa*. Hydrazines **2h**, **2k**, and **2p**, *N*-acetyloxadiazole **3a**, ethylsulfanyloxadiazole **7b**, and phenylsulfonyl hydrazine **8a** were twice as active as ampicillin against *P. aeruginosa* whereas compounds **2a**–**2c**, **2f**, **2g**, **2l**, **2o**, and **2q**, *N*-acetyloxadiazoles **3c**, **3d**, **3e**, **3g** and benzylsulfanyloxadiazole **7c** were equipotent to ampicillin (Table 2).

Compound **8b** was also active against *E. coli* (nearly equipotent to ampicillin). Only compound **2m** showed mild antifungal activity (almost half that of clotrimazole). The rest of the tested compounds were inactive against *C. albicans* (Table 2).

In vitro cytotoxicity study

The most active compounds **2h**, **2i**, and **8b** were evaluated against the normal cell line peripheral blood mononuclear cell (PBMC) to predict their safety profile using neutral red uptake assay and 5-fluorouracil (5-FU) as a reference drug. Results revealed that compounds **2h**, **2i**, and **8b** were more safe on PBMC than 5-FU. Their IC_{50} values were found to be 3.291, 7.651, and 21.216 mg/cm^3, respectively, compared to 5-FU (0.016 mg/cm^3).

In silico analysis

In silico tools (OSIRIS Property Explorer and Marvin Sketch 6.3.0) were used to predict the drug-likeness, scores, and toxicity (such as mutagenic, tumorigenic, irritant, and reproductive effects) of the active compounds (Supplementary Material, Tables 2 and 3) [43, 44]. All the compounds were predicted to be safe and hydrazines **2a**, **2c**, and **2n** had the best drug-likeliness scores.

Structure activity relationship

Generally, hydrazines exhibited better antioxidant and antimicrobial activities than 1,3,4-oxadiazoles. Incorporation of a methylphenylsulfonyl moiety (compound **8b**) to hydrazines increased the antibacterial and antioxidant potencies. Substituting the methylphenylsulfonyl group with phenylsulfonyl (compound **8a**) diminished the antimicrobial activity and maintained the moderate antioxidant potency. Incorporation of 2,3,4-trimethoxyphenyl (compound **2h**) and 2-naphthalenyl (compound **2j**) moieties to hydrazines resulted into the most potent antioxidant compounds in this study.

Conclusion

Most of the tested hydrazines and 1,3,4-oxadiazoles displayed considerable activity against *P. aeruginosa.* Methylphenylsulfonylhydrazine **8b** was almost equipotent to ampicillin against *E. coli* with moderate antioxidant activity. None of the compounds showed antifungal potency except 2-pyridinylethylidenehydrazine **2m** which exhibited mild activity.

Hydrazines bearing 2,3,4-trimethoxyphenyl (**2h**) and 2-naphthalenyl (**2j**) moieties were the most potent antioxidant derivatives, whereas 3-hydroxy-4-methoxyphenylhydrazine **2e**, phenylsulfonyl hydrazine **8a**, as well as 1,3,4-oxadiazoles bearing 3,4,5-trimethoxyphenyl (**3f**) and amino (**5**) moieties revealed moderate antioxidant activities.

In silico studies revealed that all these derivatives exhibit good drug-likeness scores and low toxicities which makes them good candidates for the design and synthesis of new similar phenolic scaffolds bearing hydrazines and 1,3,4-oxadiazoles with the aim of improving antimicrobial and antioxidant activities.

Experimental

Melting points were determined in open glass capillaries using a Griffin melting point apparatus or an Electrothermal capillary tube melting point apparatus. Infrared spectra (IR) were recorded, using KBr discs, by a Perkin-Elmer 1430 Infrared spectrophotometer, Central Laboratory, Faculty of Pharmacy, Alexandria University or by a Vertex 70 (Bruker Optics Inc., MA, USA) Fourier transform infrared spectroscopy (FTIR) instrument, Faculty of Pharmacy, University of Helsinki, Finland. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) were determined using a Mercury 300 MHz spectrometer, Faculty of Science, Cairo University, a Bruker high performance digital FT-

Table 2 Minimum inhibitory concentrations (MIC) and minimum germicidal concentrations (MBC/MFC) of tested compounds in μg/cm^3

Comp.	Gram-positive bacteria				Gram-negative bacteria				Fungus	
	S. aureus		*B. subtilis*		*E. coli*		*P aeruginosa*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
2a	100	100	100	200	25	25	50	100	50	100
2b	50	100	50	100	100	100	50	100	25	50
2c	100	100	100	100	100	200	50	50	50	100
2d	25	50	50	50	100	200	100	100	50	100
2e	100	100	50	50	25	50	100	200	100	200
2f	50	100	100	100	100	200	50	50	50	100
2g	50	100	100	200	100	100	50	100	50	100
2h	50	100	100	200	50	100	25	50	100	100
2i	50	50	50	100	50	50	100	200	100	100
2j	100	100	100	100	100	100	100	100	50	100
2k	100	100	50	50	25	50	25	25	50	100
2l	50	100	100	200	100	100	50	100	50	50
2m	100	200	100	200	50	50	100	100	12.5	25
2n	100	100	50	50	25	25	100	100	100	200
2o	100	200	25	50	25	50	50	100	100	200
2p	25	50	25	50	50	100	25	50	50	50
2q	50	100	100	100	100	100	50	100	50	50
3a	100	100	100	100	50	100	25	50	50	100
3b	100	100	50	100	100	100	100	100	50	50
3c	100	200	50	50	25	50	50	100	100	200
3d	50	100	100	100	100	100	50	100	50	100
3e	25	50	25	50	50	100	50	50	50	100
3f	100	100	100	200	50	50	100	100	25	50
3g	100	100	100	200	25	50	50	100	50	100
5	25	50	25	100	50	50	100	200	100	100
6	25	50	50	50	100	100	100	200	100	100
7a	100	100	100	200	100	100	100	100	50	100
7b	100	200	100	100	25	50	25	50	100	100
7c	100	100	100	200	25	50	50	100	50	100
8a	50	100	100	100	50	100	25	50	25	50
8b	100	200	50	50	12.5	25	12.5	25	50	100
A[a]	5	–	12.5	–	10	–	50	–	–	–
C[b]	–	–	–	–	–	–	–	–	5	–

[a] *A* ampicillin trihydrate (standard broad spectrum antibiotic)

[b] *C* clotrimazole (standard broad spectrum antifungal agent)

NMR 400 MHz spectrometer, Faculty of Pharmacy, Cairo University, or a Varian Mercury Plus 300 MHz spectrometer, Faculty of Pharmacy, University of Helsinki, Finland in DMSO-d_6 and are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. The type of signal was indicated by one of the following letters: s = singlet, d = doublet, t = triplet, q = quardruplet and m = multiplet. The coupling constants J are quoted in hertz (Hz). High resolution mass spectra (HRMS) were run on a Synapt G2 HDMS (Waters, MA, USA), Faculty of Pharmacy, University of Helsinki, Finland. Elemental microanalyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University or at Robertson Microlit Laboratories Inc., Ledgewood, New Jersey, USA. Their results were found to be in good agreement (±0.3%) with the calculated values. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel (60 GF254, Merck) using glass plates

or on Kieselgel 60HF254/Kieselgel 60G TLC plates and the spots were visualized by exposure to iodine vapor or to UV-lamp at $\lambda = 254$ nm for few seconds.

2,5-Dihydroxybenzohydrazide (**1**) was prepared according to the reported method [38].

*General method for the synthesis of dihydroxybenzoyl methylene- and ethylidenehydrazines **2a–2q***

A solution of the selected aldehyde or ketone (10 mmol) in 10 cm^3 absolute ethanol was gradually added to a warm stirred solution of 1.68 g hydrazide **1** (10 mmol) in 40 cm^3 absolute ethanol and 2 cm^3 glacial acetic acid. The reaction mixture was then heated under reflux while stirring for 1 h. The formed beige precipitates were filtered, washed with cold 50% ethanol, air dried, and crystallized from the appropriate solvent.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(1H-imidazol-4-yl-methylene)hydrazine (**2a**, $C_{11}H_{10}N_4O_3$)

Crystallized from methanol. Yield 83%; m.p.: 286–287 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 6.79$ (d, 1H, $J = 8.7$ Hz), 6.90 (dd, 1H, $J = 8.7$, 2.7 Hz), 7.18 (d, 1H, $J = 2.7$ Hz), 7.52 (s, 1H), 7.74 (s, 1H), 8.04 (s, 1H, CH=N), 9.13, 11.33 (2 s, each 1H, 2OH), 12.82, 14.27 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): $\delta = 112.8$, 115.6, 118.7, 122.1, 122.3, 135.9, 136.8, 137.0, 149.9, 153.1, 165.4 ppm; IR (ATR): $\bar{\nu} = 3150$ (OH), 3100 (υNH), 1659 (C=O), 1597 (C=N), 1574 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(4-hydroxyphenyl-methylene)hydrazine (**2b**, $C_{14}H_{12}N_2O_4$)

Crystallized from ethanol. Yield 55%; m.p.: 247–248 °C; ^{1}H NMR (300 MHz): $\delta = 6.80$ (d, 2H, $J = 8.7$ Hz), 6.84 (d, 1H, $J = 8.7$ Hz), 6.89 (dd, 1H, $J = 8.7$, 3 Hz), 7.30 (d, 1H, $J = 3$ Hz), 7.57 (d, 2H, $J = 8.7$ Hz), 8.34 (s, 1H, CH=N), 9.06, 9.92, 11.18 (3 s, each 1H, 3OH), 11.60 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz): $\delta = 114.4$, 116.1, 116.5, 118.2, 121.7, 125.6, 129.4, 149.2, 149.9, 151.9, 160.0, 164.5 ppm; IR (KBr): $\bar{\nu} = 3335$ (OH), 3264 (υNH), 1637 (C=O), 1603 (C=N), 1593 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(2,3,4-trihydroxyphenyl-methylene)hydrazine (**2c**, $C_{14}H_{12}N_2O_6$)

Crystallized from methanol. Yield 86%; m.p.: 259–260 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 6.38$ (d, 1H, $J = 8.4$ Hz), 6.77 (d, 1H, $J = 8.7$ Hz), 6.80 (d, 1H, $J = 8.7$ Hz), 6.89 (dd, 1H, $J = 8.7$, 3 Hz), 7.27 (d, 1H, $J = 3$ Hz), 8.43 (s, 1H, CH=N), 8.47, 9.07, 9.43, 11.06, 11.41 (5 s, each 1H, 5OH), 11.80 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz): $\delta = 108.1$, 109.9, 111.2, 114.2, 116.1, 118.3, 121.7, 133.1, 148.0, 149.3, 150.0, 151.2, 151.9, 164.2 ppm; IR (ATR): $\bar{\nu} = 3362$ (OH), 3300 (υNH), 1651 (C=O), 1607 (C=N), 1541 (δNH) cm^{-1}; HRMS (ESI): *m/z* calcd. for $C_{14}H_{13}N_2O_6$ 305.0774 ($[M + 1]^+$), found 305.0778.

*N^1-(2,5-Dihydroxybenzoyl)-N^2-(4-hydroxy-3-methoxyphenylmethylene)hydrazine (**2d**)*

Crystallized from ethanol. Yield 72%; m.p.: 267–268 °C (Chaaban [45] 267–268 °C).

N^1-(2,5-Dihydroxybenzoyl)-N^2-(3-hydroxy-4-methoxyphenylmethylene)hydrazine (**2e**, $C_{15}H_{14}N_2O_5$)

Crystallized from ethanol. Yield 92%; m.p.: 159–160 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 3.81$ (s, 3H, OCH_3), 6.80 (d, 1H, $J = 8.7$ Hz), 6.89 (dd, 1H, $J = 8.7$, 2.1 Hz), 6.98 (d, 1H, $J = 8.1$ Hz), 7.08 (d, 1H, $J = 8.4$ Hz), 7.27–7.30 (m, 2H), 8.29 (s, 1H, CH=N), 9.06, 9.27, 11.14 (3 s, each 1H, 3OH), 11.60 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz): $\delta = 56.1$, 112.3, 112.8, 114.4, 116.5, 118.2, 120.8, 121.7, 127.4, 147.3, 149.1, 149.9, 150.4, 151.9, 164.6 ppm; IR (KBr): $\bar{\nu} = 3187$ (OH), 3125 (υNH), 1632 (C=O), 1601 (C=N), 1567 (δNH), 1225, 1036 (C–O–C) cm^{-1}.

*N^1-(2,5-Dihydroxybenzoyl)-N^2-(3-ethoxy-4-hydroxyphenyl-methylene)hydrazine (**2f**)*

Crystallized from ethanol. Yield 70%; m.p.: 235–236 °C (Chaaban [45] 235–236 °C).

N^1-(2,5-Dihydroxybenzoyl)-N^2-(3,4-dimethoxyphenyl-methylene)hydrazine (**2g**, $C_{16}H_{16}N_2O_5$)

Crystallized from methanol. Yield 69%; m.p.: 229–230 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 3.81$, 3.82 (2 s, each 3H, $2OCH_3$), 6.81 (d, 1H, $J = 8.7$ Hz), 6.90 (dd, 1H, $J = 8.85$, 2.1 Hz), 7.03 (d, 1H, $J = 8.1$ Hz), 7.22 (d, 1H, $J = 8.1$ Hz), 7.31 (d, 1H, $J = 2.4$ Hz), 7.35 (s, 1H), 8.37 (s, 1H, CH=N), 9.07, 11.11 (2 s, each 1H, 2OH), 11.66 (s, 1H, NH) ppm; IR (KBr): $\bar{\nu} = 3274$ (OH), 3200 (υNH), 1646 (C=O), 1602 (C=N), 1544 (δNH), 1228, 1015 (C–O–C) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(2,3,4-trimethoxyphenyl-methylene)hydrazine (**2h**, $C_{17}H_{18}N_2O_6$)

Crystallized from methanol. Yield 93%; m.p.: 198–199 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 3.76$, 3.83, 3.84 (3 s, each 3H, $3OCH_3$), 6.78 (d, 1H, $J = 8.7$ Hz), 6.88–6.93 (m, 2H), 7.30 (d, 1H, $J = 2.7$ Hz), 7.61 (d, 1H, $J = 9$ Hz), 8.61 (s, 1H, CH=N), 9.05, 11.22 (2 s, each 1H, 2OH), 11.73 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz): $\delta = 56.4$, 60.9, 62.2, 109.1, 114.0, 116.1, 118.3, 120.6, 121.1, 121.9, 142.0, 144.7, 149.8, 152.4, 153.2, 155.7, 165.1 ppm; IR (ATR): $\bar{\nu} = 3400$ (OH), 3242 (υNH), 1632 (C=O), 1612 (C=N), 1583 (δNH), 1234, 1038 (C–O–C) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(3,4,5-trimethoxyphenyl-methylene)hydrazine (**2i**, $C_{17}H_{18}N_2O_6$)

Crystallized from methanol. Yield 88%; m.p.: 247–248 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 3.72$, 3.84, 3.90 (3 s, each 3H, $3OCH_3$), 6.81 (d, 1H, $J = 8.85$ Hz), 6.90 (dd, 1H, $J = 8.85$, 2.7 Hz), 7.04 (s, 2H), 7.30 (d, 1H, $J = 2.7$ Hz), 8.37 (s, 1H, CH=N), 9.08, 11.03 (3 s, each 1H, 3OH),

11.73 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz): δ = 56.4, 60.6, 104.8, 114.6, 116.9, 118.3, 121.7, 130.1, 139.7, 148.8, 150.0, 151.5, 153.6, 164.4 ppm; IR (KBr): $\bar{\nu}$ = 3346 (OH), 3285 (υNH), 1631 (C=O), 1605 (C=N), 1562 (δNH), 1232, 1062 (C–O–C) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(2-naphthalenylmethylene)hydrazine (**2j**, $C_{18}H_{14}N_2O_3$)
Crystallized from methanol. Yield 66%; m.p.: 226–227 °C (decomp.); ^{1}H NMR (300 MHz): δ = 6.81 (d, 1H, J = 8.7 Hz), 6.89 (dd, 1H, J = 8.85, 2.85 Hz), 7.31 (d, 1H, J = 2.7 Hz), 7.53–7.59 (m, 2H), 7.92–8.01 (m, 4H), 8.15 (s, 1H), 8.60 (s, 1H, CH=N), 9.07, 11.10 (2 s, each 1H, 2OH), 11.81 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz): δ = 114.5, 116.7, 118.3, 121.8, 123.2, 127.2, 127.6, 128.2, 128.8, 128.9, 129.3, 132.4, 133.2, 134.2, 148.8, 150.0, 151.7, 164.7 ppm; IR (ATR): $\bar{\nu}$ = 3262 (OH), 3159 (υNH), 1628 (C=O), 1603 (C=N), 1549 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(2-hydroxy-1-naphthalenylmethylene)hydrazine (**2k**, $C_{18}H_{14}N_2O_4$)
Crystallized from methanol. Yield 51%; m.p.: 247–248 °C (decomp.); ^{1}H NMR (300 MHz): δ = 6.86 (d, 1H, J = 8.7 Hz), 6.93 (dd, 1H, J = 8.4, 2.4 Hz), 7.27 (d, 1H, J = 9 Hz), 7.34 (d, 1H, J = 2.7 Hz), 7.41 (t, 1H, J = 7.35 Hz), 7.60 (t, 1H, J = 7.65 Hz), 7.89 (d, 1H, J = 8.1 Hz), 7.94 (d, 1H, J = 9 Hz), 8.30 (d, 1H, J = 8.4 Hz), 9.14 (s, 1H, CH=N), 9.55, 10.99, 12.01 (3 s, each 1H, 3OH), 12.79 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz): δ = 109.0, 114.6, 116.4, 118.4, 119.4, 121.4, 122.1, 124.0, 128.1, 128.2, 129.4, 132.1, 133.3, 147.9, 150.1, 151.6, 158.5, 164.0 ppm; IR (KBr): $\bar{\nu}$ = 3189 (OH), 3053 (υNH), 1643 (C=O), 1599 (C=N), 1545 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(5-bromo-3-indolylmethylene)hydrazine (**2l**, $C_{16}H_{12}BrN_3O_3$)
Crystallized from methanol. Yield 94%; m.p.: 277–278 °C (decomp.); ^{1}H NMR (300 MHz): δ = 6.79 (d, 1H, J = 8.7 Hz), 6.88 (dd, 1H, J = 8.85, 2.85 Hz), 7.30–7.34 (m, 2H), 7.42 (d, 1H, J = 8.7 Hz), 7.88 (d, 1H, J = 2.7 Hz), 8.44 (d, 1H, J = 1.8 Hz), 8.58 (s, 1H, CH=N), 9.03, 11.29 (2 s, each 1H, 2OH), 11.55, 11.77 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): δ = 111.6, 113.6, 114.1, 114.3, 116.4, 118.2, 121.6, 124.5, 125.6, 126.4, 132.4, 136.2, 145.7, 149.8, 152.2, 164.4 ppm; IR (ATR): $\bar{\nu}$ = 3123 (OH), 3071 (υNH), 1624 (C=O), 1612 (C=N), 1558 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-[1-(2-pyridinyl)ethylidene]hydrazine (**2m**, $C_{14}H_{13}N_3O_3$)
Crystallized from methanol. Yield 87%; m.p.: 259–260 °C (decomp.); ^{1}H NMR (300 MHz): δ = 2.39 (s, 3H, CH_3), 6.85–6.86 (m, 2H), 7.39–7.42 (m, 2H), 7.83–7.88 (m, 1H), 8.11 (d, 1H, J = 8.1 Hz), 8.59–8.61 (m, 1H), 9.08, 11.04 (2 s, each 1H, 2OH), 11.53 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz): δ = 12.1, 116.3, 118.2, 118.7, 120.7, 121.3, 124.4, 137.0, 149.0, 149.1, 150.7, 152.1, 155.3, 162.0 ppm; IR (ATR): $\bar{\nu}$ = 3277 (OH), 3165 (υNH), 1649 (C=O), 1626 (C=N), 1587 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-[1-(2-pyrazinyl)ethylidene]hydrazine (**2n**, $C_{13}H_{12}N_4O_3$)
Crystallized from methanol. Yield 91%; m.p.: 268–269 °C (decomp.); ^{1}H NMR (300 MHz): δ = 2.37 (s, 3H, CH_3), 6.86 (m, 2H), 7.41 (s, 1H), 8.62–8.66 (m, 2H), 9.11 (s, 1H), 9.26, 11.09 (2 s, each 1H, 2OH), 11.63 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz): δ = 11.9, 109.9, 116.2, 118.2, 118.5, 121.5, 142.7, 143.7, 144.5, 149.1, 150.3, 150.8, 162.0 ppm; IR (ATR): $\bar{\nu}$ = 3246 (OH), 3155 (υNH), 1661 (C=O), 1601 (C=N), 1552 (δNH) cm^{-1}.

N^1-(2,5-Dihydroxybenzoyl)-N^2-[1-(1-naphthalenyl)ethylidene]hydrazine (**2o**, $C_{19}H_{16}N_2O_3$)
Crystallized from methanol. Yield 94%; m.p.: 255–256 °C (decomp.); ^{1}H NMR (300 MHz): δ = 2.38 (s, 3H, CH_3), 6.46 (d, 1H, J = 8.7 Hz), 6.62 (dd, 1H, J = 8.7, 3 Hz), 7.28 (d, 1H, J = 3 Hz), 7.48–7.64 (m, 5H), 8.01–8.06 (m, 2H), 8.89, 9.53 (2 s, each 1H, 2OH), 10.78 (s, 1H, NH) ppm; IR (ATR): $\bar{\nu}$ = 3277 (OH), 3157 (υNH), 1645 (C=O), 1616 (C=N), 1587 (δNH) cm^{-1}; HRMS (ESI): *m/z* calcd. for $C_{19}H_{17}N_2O_3$ 321.1239 ($[M + 1]^+$), found 321.1242.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(2-oxoindolin-3-ylidene)hydrazine (**2p**, $C_{15}H_{11}N_3O_4$)
Crystallized from ethanol. Yield 88%; m.p.: >300 °C; ^{1}H NMR (300 MHz): δ = 6.90–6.97 (m, 3H), 7.06–7.11 (m, 1H), 7.40–7.45 (m, 2H), 7.91 (d, 1H, J = 7.8 Hz), 9.20, 10.79 (2 s, each 1H, 2OH), 11.51, 12.21 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): δ = 111.5, 115.7, 116.4, 118.0, 118.4, 122.3, 122.5, 124.0, 133.2, 138.7, 144.3, 149.0, 151.1, 163.1, 165.2 ppm; IR (ATR): $\bar{\nu}$ = 3373 (OH), 3269 (υNH), 1668 (C=O), 1618 (C=N), 1595 (δNH) cm^{-1}; HRMS (ESI): *m/z* calcd. for $C_{15}H_{12}N_3O_4$ 298.0828 ($[M + 1]^+$), found 298.0827.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(5-iodo-2-oxoindolin-3-ylidene)hydrazine (**2q**, $C_{15}H_{10}IN_3O_4$)
Crystallized from ethanol. Yield 92%; m.p.: >300 °C; ^{1}H NMR (300 MHz): δ = 6.76 (d, 1H, J = 8.4 Hz), 6.80–6.90 (m, 2H), 7.38 (d, 1H, J = 3 Hz), 7.66 (dd, 1H, J = 8.1, 1.8 Hz), 7.78 (d, 1H, J = 1.8 Hz), 9.09, 10.95 (2 s, each 1H, 2OH), 11.18, 14.31 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): δ = 85.5, 113.6, 116.4, 117.7, 118.2, 122.5, 123.4, 128.9, 135.9, 139.6, 142.2, 149.9, 150.6, 161.3, 163.2 ppm; IR (ATR): $\bar{\nu}$ = 3423 (OH), 3306 (υNH), 1632 (C=O), 1614 (C=N), 1589 (δNH) cm^{-1}.

*General method for the synthesis of acetoxyoxadiazolylphenyl acetates **3a–3g***
Amixture of the appropriate hydrazone **2b**, **2d–2g**, **2i**, **2k** (1 mmol) in 5 cm^3 acetic anhydride was heated under

reflux for 2 h. The reaction mixture was then concentrated to half its volume under reduced pressure and set aside to attain room temperature. The reaction mixture was gradually poured onto stirred crushed ice to decompose the excess unreacted acetic anhydride and left in a refrigerator for an overnight. The separated products were filtered, washed with water, air dried, and crystallized from the selected solvent.

4-Acetoxy-2-[4-acetyl-5-(4-acetoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3a**, $C_{22}H_{20}N_2O_8$) Crystallized from ethanol. Yield 57%; m.p.: 147–148 °C; ^{1}H NMR (300 MHz): $\delta = 2.25$ (s, 3H, $COCH_3$), 2.26, 2.27 (2 s, 9H, $3OCOCH_3$), 7.16 (s, 1H), 7.21 (d, 2H, $J = 8.7$ Hz), 7.36 (d, 1H, $J = 9$ Hz), 7.41 (dd, 1H, $J = 9$, 2.7 Hz), 7.50 (d, 2H, $J = 8.4$ Hz), 7.60 (d, 1H, $J = 2.7$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 21.1$, 21.2, 21.3, 21.7, 91.1, 118.8, 122.8, 122.9, 126.0, 126.8, 128.5, 134.2, 146.3, 148.5, 151.5, 152.0, 167.3, 169.4, 169.5, 169.7 ppm; IR (KBr): $\bar{\nu} = 1765$ (C=O ester), 1661 (C=O amide), 1616 (C=N), 1209, 1048 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(4-acetoxy-3-methoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3b**, $C_{23}H_{22}N_2O_9$) Crystallized from ethanol. Yield 71%; m.p.: 155–156 °C; ^{1}H NMR (300 MHz): $\delta = 2.23$ (s, 3H, $COCH_3$), 2.25, 2.26 (2 s, 9H, $3OCOCH_3$), 3.79 (s, 3H, OCH_3), 7.09 (s, 1H), 7.16 (d, 1H, $J = 8.1$ Hz), 7.20 (d, 1H, $J = 2.7$ Hz), 7.33 (dd, 1H, $J = 8.1$, 2.7 Hz), 7.37 (d, 1H, $J = 8.7$ Hz), 7.41 (dd, 1H, $J = 8.7$, 2.7 Hz), 7.58 (d, 1H, $J = 2.4$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 20.8$, 21.1, 21.2, 21.8, 56.4, 91.2, 113.3, 118.8, 121.5, 122.8, 125.8, 125.9, 126.8, 129.2, 139.8, 146.3, 148.5, 151.4, 152.6, 167.3, 168.9, 169.5, 169.6 ppm; IR (KBr): $\bar{\nu} = 1764$ (C=O ester), 1664 (C=O amide), 1619 (C=N), 1212, 1051 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(3-acetoxy-4-methoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3c**, $C_{23}H_{22}N_2O_9$) Crystallized from methanol. Yield 83%; m.p.: 135–136 °C; ^{1}H NMR (300 MHz): $\delta = 2.23$ (s, 3H, $COCH_3$), 2.26, 2.27 (2 s, 9H, $3OCOCH_3$), 3.79 (s, 3H, OCH_3), 7.02 (dd, 1H, $J = 8.1$, 1.8 Hz), 7.13 (s, 1H), 7.16 (d, 1H, $J = 8.1$ Hz), 7.21 (d, 1H, $J = 1.5$ Hz), 7.35 (d, 1H, $J = 8.7$ Hz), 7.41 (dd, 1H, $J = 8.7$, 2.7 Hz), 7.60 (d, 1H, $J = 2.7$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 20.8$, 21.1, 21.2, 21.8, 56.3, 91.4, 111.6, 118.8, 119.1, 122.8, 123.9, 125.7, 127.0, 135.7, 140.9, 146.4, 148.5, 151.5, 151.6, 167.3, 168.9, 169.5, 169.6 ppm; IR (KBr): $\bar{\nu} = 1763$ (C=O ester), 1670 (C=O amide), 1625 (C=N), 1208, 1041 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(4-acetoxy-3-ethoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3d**, $C_{24}H_{24}N_2O_9$) Crystallized from ethanol. Yield 85%; m.p.: 165–166 °C; ^{1}H NMR (300 MHz): $\delta = 1.28$ (t, 3H, $J = 6.9$ Hz, OCH_2CH_3), 2.23 (s, 3H, $COCH_3$), 2.25, 2.26 (2 s, 9H, $OCOCH_3$), 3.79 (s, 3H, OCH_3), 4.05 (q, 2H, $J = 6.9$ Hz, OCH_2CH_3), 7.00 (dd, 1H, $J = 8.1$, 1.8 Hz), 7.12 (s, 1H), 7.15 (d, 1H, $J = 8.1$ Hz), 7.19 (d, 1H, $J = 1.8$ Hz), 7.35 (d, 1H, $J = 8.7$ Hz), 7.41 (dd, 1H, $J = 8.7$, 2.7 Hz), 7.60 (d, 1H, $J = 2.7$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 14.8$, 20.7, 21.1, 21.2, 21.8, 64.7, 91.4, 112.6, 118.7, 119.0, 122.8, 123.7, 125.7, 126.8, 135.4, 141.2, 146.3, 148.6, 150.7, 151.6, 167.3, 168.8, 169.5, 169.7 ppm; IR (KBr): $\bar{\nu} = 1762$ (C=O ester), 1670 (C=O amide), 1625 (C=N), 1210, 1044 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3e**, $C_{22}H_{22}N_2O_8$) Crystallized from ethanol. Yield 80%; m.p.: 115–116 °C; ^{1}H NMR (300 MHz): $\delta = 2.23$ (s, 3H, $COCH_3$), 2.25, 2.26 (2 s, each 3H, $2OCOCH_3$), 3.76, 3.77 (s, 3H, $2OCH_3$), 6.96 (d, 1H, $J = 9$ Hz), 7.00 (s, 1H), 7.01 (d, 1H, $J = 8.1$ Hz), 7.06 (s, 1H), 7.35 (d, 1H, $J = 8.7$ Hz), 7.40 (dd, 1H, $J = 8.7$, 2.4 Hz), 7.58 (d, 1H, $J = 2.4$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 21.1$, 21.2, 21.8, 55.9, 56.0, 91.9, 110.3, 112.1, 119.1, 119.6, 122.6, 125.7, 126.7, 129.1, 146.3, 148.5, 149.3, 150.5, 151.5, 167.1, 169.5, 169.6 ppm; IR (KBr): $\bar{\nu} = 1762$ (C=O ester), 1672 (C=O amide), 1612 (C=N), 1207, 1023 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3f**, $C_{23}H_{24}N_2O_9$) Crystallized from methanol. Yield 85%; m.p.: 131–132 °C; ^{1}H NMR (300 MHz): $\delta = 2.22$ (s, 3H, $COCH_3$), 2.27 (s, 6H, $2OCOCH_3$), 3.67, 3.78 (2 s, 9H, $3OCH_3$), 7.06 (s, 1H), 6.74 (s, 2H), 7.35 (d, 1H, $J = 8.7$ Hz), 7.41 (dd, 1H, $J = 8.7$, 2.7 Hz), 7.59 (d, 1H, $J = 2.7$ Hz) ppm; ^{13}C NMR (100 MHz): $\delta = 21.0$, 21.2, 21.8, 56.4, 60.4, 92.0, 104.3, 118.9, 122.8, 125.6, 126.8, 132.2, 139.1, 146.3, 148.5, 151.7, 153.6, 167.3, 169.5, 169.6 ppm; IR (KBr): $\bar{\nu} = 1770$ (C=O ester), 1664 (C=O amide), 1625 (C=N), 1209, 1053 (C–O–C) cm^{-1}.

4-Acetoxy-2-[4-acetyl-5-(2-acetoxy-1-naphthalenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]phenyl acetate (**3g**, $C_{26}H_{22}N_2O_8$) Crystallized from ethanol. Yield 82%; m.p.: 247–248 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 2.16$ (s, 3H, $COCH_3$), 2.19, 2.22, 2.32 (3 s, each 3H, $3OCOCH_3$), 3.79 (s, 3H, OCH_3), 7.35 (d, 1H, $J = 8.7$ Hz), 7.41 (s, 1H), 7.42 (d, 1H, $J = 2.1$ Hz), 7.55–7.68 (m, 3H), 8.02–8.05 (m, 2H), 8.09 (d, 1H, $J = 9$ Hz), 8.30–8.40 (m, 1H) ppm; ^{13}C NMR (100 MHz): $\delta = 21.0$, 21.1, 21.2, 21.5, 86.4,

119.1, 121.8, 122.7, 123.0, 123.3, 126.0, 126.4, 126.8, 128.1, 129.2, 131.6, 131.8, 132.1, 146.3, 148.6, 149.1, 151.5, 166.6, 169.4, 169.5, 169.6 ppm; IR (KBr): $\bar{\nu} = 1764$ (C=O ester), 1676 (C=O amide), 1624 (C=N), 1200, 1042 (C–O–C) cm^{-1}.

2-(5-Amino-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (**5**, $C_8H_7N_3O_3$)

A stirred suspension of 5.04 g 2,5-dihydroxybenzohydrazide (1, 30 mmol) and 5.83 g potassium thiocyanate (60 mmol) in 50 cm^3 ethanol containing six drops of concentrated hydrochloric acid was heated under reflux for 24 h. The reaction mixture was then concentrated and left to attain room temperature. The separated white product was then filtered and air dried. A second crop was also collected by evaporating the filtrate to dryness under reduced pressure. The product potassium 1-(2,5-dihydroxybenzoyl)thiosemicarbazide (4) thus obtained was pure enough to be used for the next step. Yield 7.2 g (90%).

A stirred suspension of 2.65 g potassium 1-(2,5-dihydroxybenzoyl)thiosemicarbazide (**4**, 10 mmol) and 2.17 g freshly prepared yellow mercuric oxide (10 mmol) in 20 cm^3 absolute ethanol was heated under reflux for 4 h. The reaction mixture was then filtered while hot and the filtrate was concentrated and allowed to attain room temperature. The separated product was filtered and air dried to yield the target compound **5** which was then crystallized from methanol as beige crystals. Yield 1 g (52%); m.p.: 261–262 °C; ^{1}H NMR (300 MHz): $\delta = 6.79$ (dd, 1H, $J = 9$, 3 Hz), 6.85 (d, 1H, $J = 9$ Hz), 6.93 (d, 1H, $J = 3$ Hz), 7.35 (s, 2H, NH_2), 9.17, 9.46 (2 s, each 1H, 2OH) ppm; ^{13}C NMR (75 MHz): $\delta = 109.4$, 111.3, 117.9, 119.8, 148.9, 150.4, 157.7, 163.4 ppm; IR (ATR): $\bar{\nu} = 3416$ (OH), 3335, 3273 (NH_2), 1632 (C=N), 1591 (δNH), 1225, 1051 (C–O–C) cm^{-1}.

2-(5-Sulfanyl-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (**6**, $C_8H_6N_2O_3S$)

Carbon disulfide (5 cm^3) was added to a solution of 1.68 g 2,5-dihydroxybenzohydrazide (**1**, 10 mmol) and 1.68 g potassium hydroxide (30 mmol) in 20 cm^3 ethanol. The reaction mixture was then heated under reflux for 5 h, cooled, treated with 30 cm^3 water and acidified with concentrated hydrochloric acid to pH 5. The reaction mixture was set aside for an overnight in a refrigerator for complete precipitation. The resulting fluffy white precipitate was then filtered, washed with cold water, air dried, and crystallized from water as fluffy white crystals. Yield 2 g (95%); m.p.: 201–202 °C; ^{1}H NMR (300 MHz): $\delta = 6.85$–6.86 (m, 2H), 6.99–7.00 (m, 1H), 9.17, 9.57 (2 s, each 1H, 2OH), 14.59 (s, 1H, SH) ppm; ^{13}C NMR (75 MHz): $\delta = 109.5$, 114.0, 118.6, 121.4, 149.4, 150.2, 160.2, 177.4 ppm; IR (ATR): $\bar{\nu} = 3424$ (OH), 2980 (SH), 1643 (C=N), 1219, 1042 (C–O–C) cm^{-1}.

*General method for the synthesis of substituted sulfanyl-oxadiazoles **7a–7c***

To a stirred mixture of 0.42 g 2-(2,5-dihydroxyphenyl)-5-sulfanyl-1,3,4-oxadiazole (**6**, 2 mmol) and 0.83 g anhydrous potassium carbonate (6 mmol) in 10 cm^3 dry acetone was added the appropriate alkyl or aralkyl halide (2 mmol). Stirring was continued at room temperature for 2 h. The reaction mixture was then treated with 30 cm^3 water, acidified with concentrated hydrochloric acid to pH 5, and kept in refrigerator overnight for complete precipitation. The formed yellow precipitates were filtered, washed with water several times, air dried, and crystallized from ethanol.

2-(5-Methylsulfanyl-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (**7a**, $C_9H_8N_2O_3S$)

Yield 78%; m.p.: 221–222 °C; ^{1}H NMR (300 MHz): $\delta = 2.74$ (s, 3H, SCH_3), 6.87–6.88 (m, 2H), 7.09–7.10 (m, 1H), 9.21, 9.43 (2 s, each 1H, 2OH) ppm; ^{13}C NMR (75 MHz): $\delta = 14.7$, 109.5, 113.5, 118.5, 121.4, 149.3, 150.4, 164.4, 164.9 ppm; IR (ATR): $\bar{\nu} = 3186$ (OH), 1626 (C=N), 1284, 1190 (C–S–C), 1215, 1032 (C–O–C) cm^{-1}.

2-(5-Ethylsulfanyl-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (**7b**, $C_{10}H_{10}N_2O_3S$)

Yield 73%; m.p.: 153–154 °C; ^{1}H NMR (300 MHz): $\delta = 1.41$ (t, 3H, $J = 6$ Hz, SCH_2CH_3), 3.28 (q, 2H, $J = 6$ Hz, SCH_2CH_3), 6.84–6.88 (m, 2H), 7.09–7.10 (m, 1H), 9.21, 9.46 (2 s, each 1H, OH) ppm; IR (ATR): $\bar{\nu} = 3188$ (OH), 1630 (C=N), 1263, 1188 (C–S–C), 1223, 1032 (C–O–C) cm^{-1}; HRMS (ESI): *m/z* calcd. for $C_{10}H_{11}N_2O_3S$ 239.0490 ($[M + 1]^+$), found 239.0490.

2-(5-Benzylsulfanyl-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (**7c**, $C_{15}H_{12}N_2O_3S$)

Yield 78%; m.p.: 181–182 °C; ^{1}H NMR (300 MHz): $\delta = 4.54$ (s, 2H, CH_2), 6.88–6.89 (m, 2H), 7.08–7.10 (m, 1H), 7.24–7.48 (m, 5H), 9.21, 9.45 (2 s, each 1H, 2OH) ppm; ^{13}C NMR (100 MHz): $\delta = 36.3$, 109.5, 113.6, 118.6, 121.5, 128.2, 129.0, 129.5, 137.0, 149.3, 150.4, 163.0, 165.1 ppm; IR (ATR): $\bar{\nu} = 3192$ (OH), 1632 (C=N), 1265, 1186 (C–S–C), 1223, 1026 (C–O–C) cm^{-1}.

*General method for the synthesis of sulfonylhydrazines **8a, 8b***

Benzenesulfonyl chloride or *p*-toluenesulfonyl chloride (10 mmol) was added to a solution of 1.68 g acid hydrazide **1** (10 mmol) in 20 cm^3 glacial acetic acid. The reaction mixture was stirred at room temperature for 24 h. The formed beige precipitates were filtered,

washed with ethanol, air dried, and crystallized from the proper solvent.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(4-phenylsulfonyl)hydrazine (**8a**, $C_{13}H_{12}N_2O_5S$)
Crystallized from ethyl acetate. Yield 44%; m.p.: 219–220 °C (decomp.); ^{1}H NMR (300 MHz): $\delta = 6.72$ (d, 1H, $J = 8.7$ Hz), 6.83 (dd, 1H, $J = 9$, 3 Hz), 7.09 (d, 1H, $J = 3$ Hz), 7.52–7.67 (m, 3H), 7.81–7.84 (m, 2H), 9.00, 10.05 (2 s, each 1H, 2OH), 10.34, 10.62 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): $\delta = 114.6$, 115.7, 118.1, 122.0, 128.0, 129.3, 133.5, 139.2, 150.0, 151.0, 166.7 ppm; IR (ATR): $\bar{\nu} = 3429$ (OH), 3348 (υNH), 1651 (C=O), 1545 (δNH), 1348, 1171 (SO_2) cm^{-1}; HRMS (ESI): *m/z* calcd. for $C_{13}H_{13}N_2O_5S$ 309.0545 ($[M + 1]^+$), found 309.0549.

N^1-(2,5-Dihydroxybenzoyl)-N^2-(4-methylphenylsulfonyl)hydrazine (**8b**, $C_{14}H_{14}N_2O_5S$)
Crystallized from methanol. Yield 67%; m.p.: 197–198 °C; ^{1}H NMR (300 MHz): $\delta = 2.07$ (s, 3H, CH_3), 6.71 (d, 1H, $J = 8.7$ Hz), 6.79–6.80 (m, 1H), 7.07 (d, 1H, $J = 2.7$ Hz), 7.34 (d, 2H, $J = 8.1$ Hz), 7.70 (d, 2H, $J = 8.4$ Hz), 9.00, 9.92 (2 s, each 1H, 2OH), 10.29, 10.60 (2 s, each 1H, 2NH) ppm; ^{13}C NMR (75 MHz): $\delta = 21.4$, 114.6, 115.8, 118.1, 121.9, 128.0, 129.8, 136.4, 143.8, 150.0, 150.9, 166.6 ppm; IR (ATR): $\bar{\nu} = 3338$ (OH), 3309 (υNH), 1655 (C=O), 1541 (δNH), 1336, 1155 (SO_2) cm^{-1}.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of compounds **2c**–**2e**, **2h**–**2j**, **2m**, **2p**, **2q**, **3a**, **3b**, **3f**, **7a**, **7c**, **5**, **8a**, and **8b** was measured from the bleaching of the colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [39]. The spectrophotometric assay uses the stable radical DPPH as a reagent. To 4 cm^3 of 0.004% (w/v) methanol solution of DPPH was added 1 cm^3 of various concentrations of the test compounds (0.25, 0.5, 0.75, and 1 mg/cm^3) in methanol. After a 30-min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition of free radical production from DPPH was calculated by the following equation:

$$\text{DPPH } \% \text{ of scavenging} = (A_c - A_s)/A_c \times 100,$$

where A_c is the absorbance of control (DPPH radical solution in methanol) and A_s is the absorbance of the sample (solution of DPPH radical and tested compound in methanol). Vitamin C was used as reference standard antioxidant. The experiments were performed using four different concentrations in triplicates. The results are expressed as mean ± standard deviation (SD) and are recorded in Table 1 and Fig. 1.

Antimicrobial screening

Compounds **2a**–**2q**, **3a**–**3g**, **5**, **6**, **7a**–**7c**, **8a**, and **8b** were evaluated for their in vitro antimicrobial activity by measuring the inhibition zones using the cup-diffusion technique [46]. Each 100 cm^3 of sterile molten agar at 45 °C received 1 cm^3 of a 6-h broth culture and then the seeded agar was poured into sterile petri dishes. Cups (8 mm in diameter) were cut in the agar and each cup received 0.1 cm^3 of the 1 mg/cm^3 solution of the test compounds. The plates were then incubated at 37 °C for 24 h or in case of *C. albicans* for 48 h. A control using DMSO without the test compound was included for each organism. The test organisms were *S. aureus* (DSM 1104) and *B. subtilis* (ATCC 6633) as Gram-positive bacteria, *E. coli* (ATCC 11775) and *P. aeruginosa* (ATCC 10145) as Gram-negative bacteria. They were also evaluated for their in vitro antifungal activity against *C. albicans* (DSM 70014). Ampicillin was used as standard antibacterial while clotrimazole was used as antifungal reference (Supplementary Material, Table 1).

Measurement of antimicrobial activities

Compounds were also further evaluated to determine their minimum inhibitory concentration (MIC) and minimum germicidal concentration (MBC/MFC) using the two-fold serial dilution method [47]. Ampicillin was used as standard antibiotic while clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as blank and showed no antimicrobial activity.

Minimum inhibitory concentration (MIC) measurement

The minimum inhibitory concentrations (MIC) of the tested compounds were measured using the two-fold serial broth dilution method. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37 °C. Two-fold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 μg/cm^3. The tubes were then inoculated with the test organisms; each 5 cm^3 received 0.1 cm^3 of the above inoculum and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the tested compounds are listed in Table 2.

Minimum germicidal concentration (MBC/MFC) measurement

MIC tests were extended to measure the MBC or MFC as follows: a loop-full from the tube not showing visible

growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC or MFC of that compound for the respective test organism. The MBC/MFC values of the tested compounds are listed in Table 2.

In vitro cytotoxicity study

PBMCs were isolated from the whole blood of a healthy volunteer by density gradient centrifugation technique as described by Boyum [48]. All cells were maintained at 37 °C in a humidified air incubator containing 5% CO_2. PBMCs cells were grown in RPMI-1640 medium. The media were supplemented with 10% heat-inactivated FBS, 200 μM L-glutamine and 25 μM HEPES to form the culture media. The seeding cell density was 3×10^3 cells/well for MCF-7 and HepG2 cells, 4×10^3 cells/well for A549 and CaCo-2 cells, and 1×10^5 cells/well for PBMCs thus 100 mm^3 of the respective culture media containing the chosen number of cells were pipetted into wells of polystyrene 96-well plates. Cells were left to adhere on the plate wells at 37 °C. Next, 100 mm^3 of different concentrations of the test compounds (10, 5, 2.5, 1.25, and 0.625 mg/cm^3) in culture media were added to the wells. 5-FU was used as a positive control. Blank wells contained 200 mm^3 of culture media only. The plate was then gently shaken and incubated at 37 °C, 5% CO_2 for 72 h. After incubation, neutral red uptake assay was used for identification of viable cells [49]. First, cells supernatant were removed by suction, centrifugation at 2000 rpm for 10 min. Then, 100 mm^3 of neutral red solution (80 $\mu g/cm^3$) was added to each well and incubated for another 3 h. After Neutral red incubation, excessive dyes were discarded by suction, centrifugation at 2000 rpm for 10 min is needed, and the stained cells were washed three times using PBS buffer then fixed with 100 mm^3 fixation solution (0.5% formalin with 1% calcium chloride) for 1 min. Then cells were destained using 100-mm^3 destaining solution (50% ethanol with 1% glacial acetic acid) for 5 min by shaking. The stain intensity was measured using automated microplate reader spectrophotometer adjusted at 540 nm. Viable cell fraction was calculated according to the following equation:

$$\text{Cell viability}/\% = [(A_t - A_b)/(A_c - A_b)] \times 100,$$

where A_t absorbance value of test compound, A_b absorbance value of blank, A_c absorbance value of control (untreated cells). Data are expressed as the means of two determinations ± SEM. The results were interpreted to calculate the concentration causing 50% cell death (IC_{50}) using GraphPad InStat3.0 software [50].

Calculation of drug-likeness properties

Drug-likeness properties according to the Lipinski's "Rule of Five" were calculated using the Marvin Sketch 6.3.0 [44]. Drug-likeness scores were computed using OSIRIS Property Explorer [43] (Supplementary Material, Table 2).

In silico toxicity prediction

Prediction of toxicity of the compounds was performed using OSIRIS Property Explorer [43] (Supplementary Material, Table 3).

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