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ANTIMICROBIAL ACTIVITY OF NEW SYNTHESIZED [(OXADIAZOLYL)METHYL]PHENYTOIN DERIVATIVES

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Abstract: A number of substituted phenytoin derivatives in addition to their sugar hydrazones were newly synthesized. Furthermore, the corresponding derived 1,3,4-oxadiazole and their thioglycoside as well as their acyclic analogs were prepared. The antimicrobial activity of the prepared compounds was evaluated against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. The dithiohydrazone as well as oxadiazole thiole derivatives, sugar hydrazones and acyclic nucleoside analogs were the highly active compounds.

Keywords: phenytoin, oxadiazole, glycosides, sugar hydrazones, antibacterial, antifungal activity

The imidazolidine-2,4-dione, or hydantoin nucleus, is a common 5-membered ring containing a reactive cyclic urea core. This heterocycle is present in a wide range of biologically active compounds including antiarrhythmics (1) anticonvulsant (2) and antitumor (3) agents. Beside the traditional usage, of hydantoin derivatives as antiepileptic (4, 5), antiarrhythmics (6), antibacterial substance and sceletal muscle relaxant (7), hydantoins have been also developed as new drugs in the treatment of other diseases, for example, nilutamide, which was approved by the FDA in 1996 as a nonsteroidal, orally active antiandrogen in the therapy of metastatic prostate cancer (8). Hydantoins are structural units frequently encountered in naturally occurring substances, mostly of marine organisms, but also of bacteria. Examples for many alkaloids extracted from sponges or corals which contain a hydantoin moiety are the well-known aplysinopsins with cytotoxic properties (10–13), axinohydantoins from *Axinella* (14) *Hymeniacidon* (15) and *Stylotella* species inhibiting protein kinase C (16, 17), naamidinene A, a dehydrohydantoin derivative from the genus *Leucettu* (18), and mukanadin B from *Agelus* species (19). Hydantocidin is a spiro nucleoside from *Streptomyces hygroscopicus* (20, 21), which possesses herbicidal and plant growth regulatory activity due to the inhibition of adenylsuccinate synthetase (22). Among these agents, phenytoin, is a well known therapeutic drug for the treatment of epileptic seizures (23). It had been effective against electrically induced seizures in cats (24) and is still the drug of choice for the treatment of generalized tonic–clonic seizures (so-called grand mal epilepsy) and focal motor seizures (25). Phenytoin has found new applications due to the neuro- and cardioprotective properties (26, 27). On the other hand, 1,3,4-oxadiazole derivatives possess a broad spectrum of biological activity in both agrochemicals and pharmaceuticals such as antibacterial (28), antimicrobial (29), insecticidal (30), herbicidal, fungicidal (31), anti-inflammatory (32), hypoglycemic (33), hypotension characteristics (34), antiviral (35) and antitumor activities (36). In view of the above facts and as continuation of our program of identification of new candidates that may be valuable in design and synthesis of new active leads (37–42) we report in the present work the synthesis and antimicrobial activity of new phenytoin derivatives, their oxadiazolyl, glycoside and acyclic analogs.

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EXPERIMENTAL

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using silicon oil on Gallenkamp apparatus. Elemental microanalyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo, Egypt. Infrared spectra were recorded on Jasco FT/IR-330E, Fourier Transform Infrared Spectrometer at cm^{-1} scale using KBr discs.

^{1}H-NMR spectra were determined by using JEOL EX-270 or JEOL ACA500 NMR spectrometers and measured in δ scale using TMS as an internal standard. Mass spectra were measured using mass spectrometer Finnigan MAT SSQ-7000 and GCMS-QP 1000EX Shimadzu Gas Chromatography MS Spectrometer.

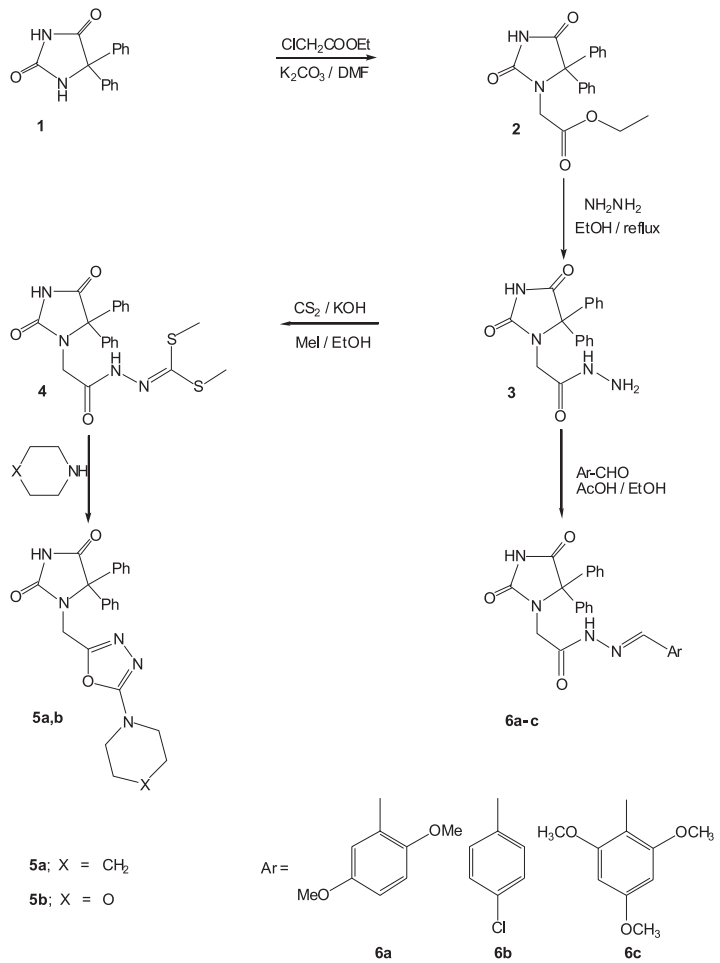
All reactions were followed up by TLC (aluminum sheets) using $CHCl_3/CH_3OH$ (9:1, v/v) eluent and detected by UV lamp. The chemical names given to the prepared compounds are according to the IUPAC system.

Ethyl 2-(phenytoin-1-yl)acetate (2)

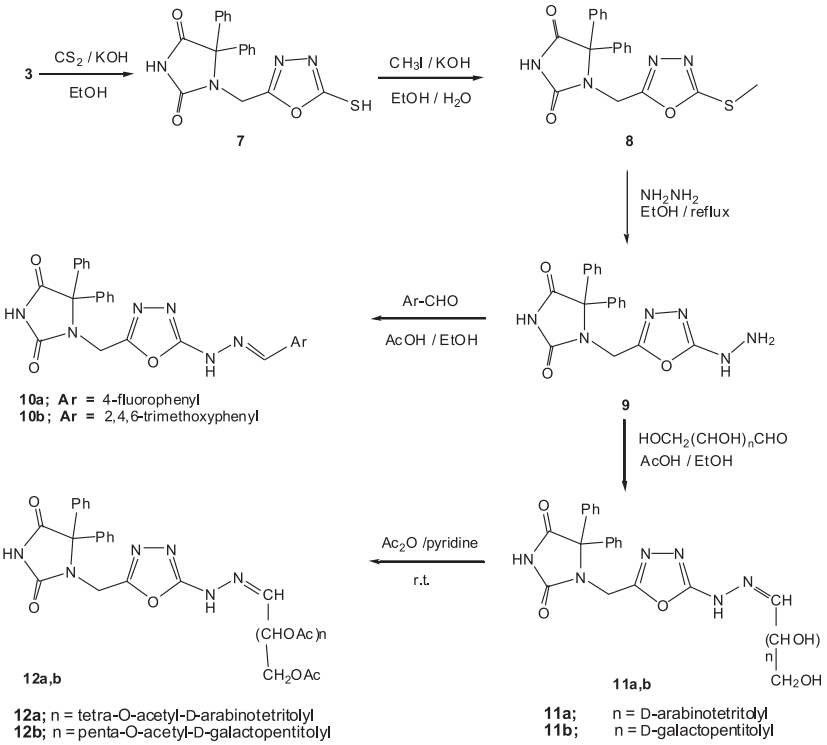
To a solution of phenytoin (5,5-diphenylhydantoin) (**1**) (2.52, 0.01 mole) in *N*,*N*-dimethyl formamide (25 mL), anhydrous potassium carbonate (0.14 g, 0.01 mole) and ethyl chloroacetate (0.12 g, 0.01 mole) were added. The solution was stirred at room temperature for 12 h and poured into ice-cold water. The resulting precipitate was filtered off and recrystallized from ethanol to afford **2**.

2-(Phenytoin-1-yl)acetohydrazide (3)

A solution of **2** (3.38 g, 0.01 mole) and hydrazine hydrate (0.5 g, 0.01 mole) in ethanol was heated under reflux for 6 h. The mixture was cooled and the precipitate was filtered off and recrystallized from ethanol to afford **3**.



Scheme 1.



Scheme 2.

Dimethyl (phenytoin-1-yl)acetyldithiohydrazonocarbonate (**4**)

To a stirred solution of potassium hydroxide (0.56 g, 0.01 mole) in 2.5 mL of water and 1.5 mL of ethanol, compound **3** (3.24 g, 0.01 mole) was added. After stirring for 1 h at room temperature, carbon disulfide (0.2 mL) and methyl iodide (0.16 mL) were added and the reaction mixture was stirred for 0.5 h. The reaction mixture was poured on ice (100 g). The yellow precipitate was filtered off and recrystallized from ethanol to afford **4**

(1,3,4-Oxadiazol-2-yl)piperidine and morpholine derivatives (**5a,b**)

Compound **4** (4.28 g, 0.01 mole) was heated under reflux in 3 mL of (morpholine or piperidine) for 2 h. The mixture was cooled, diluted with 20 mL of water and extracted with chloroform. The collected chloroform fractions were dried with magnesium sulfate. After evaporation of the solvent residual oil crystallized.

***N*'-Arylidine-2-(phenytoin-1-yl)acetohydrazide** (**6a-c**)

To solution of compound **3** (3.24 g, 0.01 mole) in ethanol, the respective aldehyde (0.01 mole) and catalytic amount of acetic anhydride were added and the reaction mixture was refluxed for 2 h. Ethanol was removed under vacuum and the obtained solid was dried well and crystallized from ethanol.

1-(5-Mercapto-[1,3,4]oxadiazol-2-ylmethyl)-5',5'-diphenyl-imidazolidine-2',4'-dione (**7**)

To a solution of **3** (3.24 g, 0.01 mole) in absolute ethanol (50 mL) a solution of potassium hydroxide (0.56 g, 0.01 mole) in water (2 mL) and carbon disulfide (5 mL) were added. The solution was heated under reflux for 20 h. The solvent was evaporated and the residue was dissolved in water, filtered, and acidified with dilute hydrochloric acid. The precipitate was filtered off, washed with water and recrystallized from ethanol.

1-(5-Methylsulfanyl-[1,3,4]oxadiazol-2-ylmethyl)-5',5'-diphenyl-imidazolidine-2',4'-dione (**8**)

To a solution of **7** (3.66 g, 0.01 mole) and potassium hydroxide (0.56 g, 0.01 mole) in a mixture of water (30 mL) and ethanol (15 mL), methyl iodide or ethyl iodide (0.01 mole) was added. The solution was stirred at room temperature for 4 h. The precipitate was filtered off and recrystallized from ethanol to afford compound **8**.

1-[(5-Hydrazino-1,3,4-oxadiazol-2-yl)methyl]-5',5'-diphenylimidazo-lidine-2',4'-dione (**9**)

A mixture of compound **8** (3.80 g, 0.01 mole) in ethanol and hydrazine hydrate (0.5 g, 0.01 mole), was refluxed for 4 h. The solvent was removed under reduced pressure, the remaining precipitate was collected, dried, and recrystallized from ethanol to afford hydrazine derivative compound.

Arylaldehyde {5-[(2',4'-dioxo-5',5'-diphenylimidazolidin-1-yl)methyl]-1,3,4-oxadiazol-2-yl}hydrazone (**10a,b**)

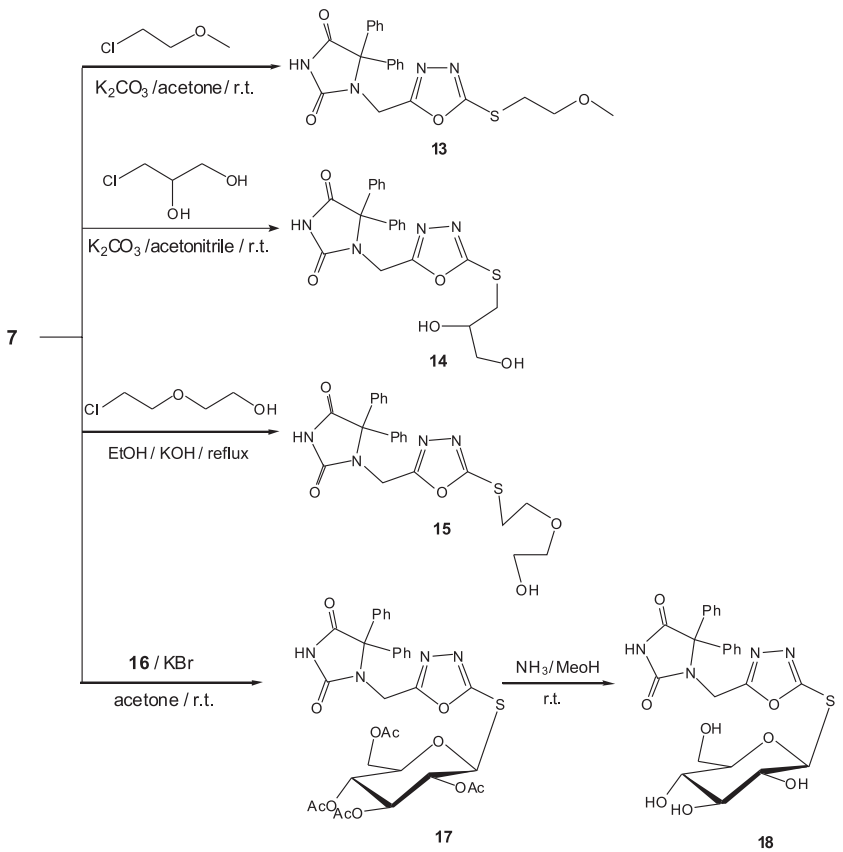
To solution of the hydrazine derivative **9** (3.64 g, 0.01 mole) in ethanol, the respective aldehyde (0.01 mole) and catalytic amount of acetic anhydride (0.5 mL) were added and the reaction mixture was refluxed for 2 h. Ethanol was removed under vacuum. The solid was dried well and recrystallized from ethanol.

Sugar {5-[(2',4'-dioxo-5',5'-diphenylimidazolidin-1-yl)methyl]-1,3,4-oxadiazol-2-yl}hydrazone (**11a**,**b**)

To a well stirred mixture of the respective monosaccharide [(0.01 mole) in water (1 mL)], glacial acetic acid (0.2 mL) in ethanol (10 mL) was added the hydrazine derivative **10** (3.64 g, 0.01 mole). The mixture was heated under reflux for 3 h and the resulting solution was concentrated and left to cool. The precipitate formed was filtered off, washed with water and ethanol then dried and crystallized from ethanol.

***O*-Acetylsugar {5-[(2',4'-dioxo-5',5'-diphenylimidazolidin-1-yl)methyl]-1,3,4-oxadiazol-2-yl}hydrazone** (**12a**,**b**)

To a solution of the hydrazinosugars **11a,b** (0.01 mole) in pyridine, acetic anhydride (0.1 mole) was added and the mixture was stirred at



Scheme 3.

room temperature for 5 h. The resulting solution was poured into crushed ice and the product that separated out was filtered off, washed with a solution of sodium hydrogen carbonate followed by water and then dried. The product was recrystallized from ethanol.

1-{[5-(Substituted alkylthio)-1,3,4-oxadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione (**13** and **14**)

General procedure: To a solution of **7** (3.66 g, 0.01 mole) in acetone or acetonitrile (15 mL), anhydrous potassium carbonate (1.38 g, 0.01 mole) was added and the mixture was stirred at room temperature for 1 h. Chloromethylethyl ether or 2,3-dihydroxypropane (0.01 mole) was added and stirring was continued for 25–30 h at room temperature and then poured into cooled water. The resulting precipitate was filtered off and recrystallized from ethanol.

1-{[5-[[2-(2-Hydroxyethoxy)ethyl]sulfanyl]-1,3,4-oxadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione (**15**)

To a solution of **7** (3.66 g, 0.01 mole) in absolute EtOH (15 mL) potassium hydroxide (0.56 g, 0.01 mole) was added and the mixture was stirred at room temperature for 1 h. 2-(2-Chloroethoxy)ethanol (1.25 g, 0.01 mole) was added and the reaction mixture was heated at reflux temperature for 6 h. The solvent was evaporated under reduced pressure and the resulting precipitate was collected and recrystallized from ethanol.

1-{[5-[(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl)thio]-1,3,4-oxadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione (**17**)

To a solution of compound **7** (0.37 g, 0.001 mole) and aqueous potassium hydroxide (1.12 g, 0.01 mole) in acetone, solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.11 g, 0.01 mole) dissolved in acetone was added. The reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure at 40°C; the residue was washed with distilled water to remove potassium bromide formed.

1-{[5-[(D-Glucopyranosyl)thio]-1,3,4-oxadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione (**18**)

A solution of **17** (0.7 g, 0.001 mole) in methanol and ammonia solution was stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure and the residue was dissolved in absolute ethanol (10 mL) and left overnight. The solvent was removed under vacuum and the product was dried well**.**

Antimicrobial screening

The synthesized compounds were screened *in vitro* for their antimicrobial activities against *Escherichia coli* NRRL B-210 (Gram negative bacteria), *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* (Gram positive bacteria), *Aspergillus niger* and *Candida albicans* NRRL Y-477 (fungi). These microorganisms were obtained from Northern Utilisation, Research and Development Division, U.S. Department of Agricultural Peoria, Illinois, USA.

The agar diffusion method reported by Cruickshank et al. (43) was used for the screening process. The bacteria and fungi were maintained on nutrient agar and Czapek's-Dox agar media, respectively.

The assay medium flasks containing 50 mL of nutrient agar for bacteria and Czapek's-Dox agar medium for fungi, respectively, were allowed to reach 40–50°C to be inoculated with 0.5 mL of the test organism cell suspension. The flasks were mixed well and poured each into a Petri dish (15 × 2 cm) and allowed to solidify. After solidification, holes (0.6 cm diameter) were made in the agar plate by the aid of a sterile cork poorer (diameter 6 mm).

The synthesized target compounds were dissolved each in 2 mL DMSO. In these holes, 100 µL of each compound was placed using an automatic micropipette. The Petri dishes were left at 5°C for 1 h to allow diffusion of the samples through the agar medium and retard the growth of the test organism. Plates were incubated at 30°C for 24 h for bacteria and 72 h of incubation at 28°C for fungi. DMSO showed no inhibition zones. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (44, 45) (50 µg/mL) and fusidic acid (46) (50 µg/mL) were used as standard for antibacterial and antifungal activity, respectively. The observed zones of inhibition are presented in Table 1.

RESULTS AND DISCUSSION

Chemistry

In this investigation, when 5,5-diphenyl hydantoin **1** was allowed to react with ethyl chloroacetate in DMF and in the presence of potassium carbonate anhydrous, the corresponding ester derivative **2** was obtained. The acid hydrazide **3** was synthesized by refluxing its corresponding ester derivative **2** and

Table 1. *In vitro* antimicrobial activity by agar diffusion method of tested compounds

Compd.	Zone of inhibition [mm]				
	Microorganisms				
	E. coli	*B. subtilis*	*S. aureus*	*A. niger*	*C. albicans*
2	–	–	14	13	–
3	–	–	8	13	14
4	19	18	16	16	17
5	17	17	16	15	–
6	19	19	18	17	19
7	19	19	18	17	18
8	–	14	15	16	15
9	19	19	18	17	17
10a	18	19	18	16	17
10b	17	17	15	16	15
11a	18	18	16	16	17
11b	19	19	18	17	18
12a	–	–	9	11	–
12b	19	18	17	16	18
13	15	15	14	13	–
14	19	18	17	16	16
15	18	17	17	15	15
17	–	15	14	13	14
18	18	17	16	15	16
Streptom.	22	22	21	–	–
Fusid.	–	–	–	17	18

Streptom. = Streptomycin; Fusid. = Fusidic acid

hydrazine hydrate in ethanol. When the hydrazide **3** reacted with carbon disulfide and methyl iodide in the presence of potassium hydroxide, it afforded the corresponding dithiohydrazonocarbonate derivative **4**. Its ^{1}H NMR spectra showed the signals of the methyl groups as two singlet signals at δ 2.42–2.49 ppm. Reaction of compound **4** with piperidine or morpholine resulted in the formation of *N*-substituted derivatives **5a,b** When hydrazide **3** was reacted with 2,5-dimethoxybenzaldehyde**,** 4-chlorobenzaldehyde or 2,4,6-trimethoxybenzaldehyde in the presence of glacial acetic acid, the corresponding arylidine derivatives **6a–c** were formed. The ^{1}H NMR spectrum of the **6c** showed the signals of the methyl groups as singlets at δ 3.73–3.87 ppm in addition to the disappearance of the NH_2 signal originally present in hydrazide **3** (Scheme 1).

When the acid hydrazide **3** was reacted with carbon disulfide in ethanol in the presence of potassium hydroxide, it afforded the oxadiazole thiol/thione **7**. Methylation of **7** with methyl iodide in alkaline medium afforded the corresponding *S*-methyl derivative **8.** Reaction of compound **8** with hydrazine hydrate gave the hydrazine derivative **9**. Its IR spectrum showed the characteristic absorption bands at 3248 cm^{-1} corresponding to the NH_2 group and 3329 cm^{-1} corresponding to the NH group. When the hydrazine derivative **9** was reacted with *p*-fluorobenzaldehyde and 2,4,6-trimethoxybenzaldehyde in the presence of glacial acetic acid, they afforded the corresponding arylidine derivatives **10a,b**. Reaction of the hydrazine derivative **9** with D-galactose and D-arabinose in an aqueous ethanolic solution and a catalytic amount of acetic acid, gave the corresponding hydrazinosugar derivatives **11a,b**, respectively. The ^{1}H NMR spectrum of **11b** revealed the H-1 signal as doublet at 7.14 ppm. Acetylation of the sugar hydrazones **11a,b** with acetic anhydride in pyridine at room temperature gave the corresponding per-*O*-acetyl derivatives

Table 2. Physical and analytical data of all new compounds.

Compd. no.	M.p. (°C)	Yield (%)	Mol. formula (Mol.wt.)	Analysis (%) calcd. / found		
				C	H	N
2	222–223	87	$C_{19}H_{18}N_2O_2$ (338.36)	67.44 67.28	5.36 5.30	8.28 8.19
3	172–174	75	$C_{17}H_{16}N_4O_3$ (324.12)	62.95 62.72	4.97 4.85	7.27 7.21
4	223–225	67	$C_{20}H_{20}N_4O_3S_2$ (428.53)	56.06 55.93	4.70 4.58	13.07 12.88
5a	172–173	60	$C_{23}H_{23}N_5O_3$ (414.18))	66.17 66.02	5.55 5.47	16.76 16.58
5b	193–194	65	$C_{22}H_{21}N_5O_4$ (419.16)	63.00 62.81	5.05 5.02	16.70 16.59
6a	173–175	86	$C_{26}H_{24}N_4O_5$ (472.49)	66.09 65.89	5.12 5.10	11.86 11.71
6b	> 300	72	$C_{24}H_{19}CLN_4O_3$ (446.89)	64.50 64.32	4.29 4.14	12.54 12.29
6c	253–255	65	$C_{27}H_{26}N_4O_6$ (502.52)	64.53 64.37	5.22 5.05	11.15 10.92
7	190–192	85	$C_{18}H_{14}N_4O_3S$ (366.08)	59.01 58.88	3.85 3.69	15.29 15.15
8	160–162	67	$C_{19}H_{16}N_4O_3S$ (380.42)	59.99 59.80	4.24 4.15	14.73 14.60
9	160–161	74	$C_{18}H_{16}N_6O_3$ (364.36)	59.34 59.15	4.43 4.29	23.07 22.93
10a	160–161	90	$C_{25}H_{19}FN_6O_3$ (470.64)	63.82 63.68	4.07 4.02	17.86 17.66
10b	250–251	87	$C_{28}H_{26}N_6O_6$ (542.54)	61.99 61.68	4.83 4.70	15.49 15.28
11a	170–172	62	$C_{25}H_{29}N_6O_8$ (541.53)	55.45 55.28	5.40 5.33	15.52 15.41
11b	168–170	65	$C_{23}H_{24}N_6O_7$ (496.47)	55.64 55.50	4.87 4.69	16.93 16.77
12a	180–182	82	$C_{34}H_{36}N_6O_{13}$ (736.68)	55.34 55.11	4.93 4.72	11.41 11.19
12b	170–171	78	$C_{31}H_{32}N_6O_{11}$ (664.62)	56.02 55.85	4.85 4.59	12.64 12.55
13	168–170	55	$C_{21}H_{20}N_4O_4S$ (424.47)	59.42 59.27	4.75 4.61	13.20 13.16
14	165–166	65	$C_{21}H_{24}N_4O_4S$ (444.05)	56.74 56.66	5.44 5.28	12.60 12.49
15	150–152	72	$C_{22}H_{22}N_4O_5S$ (454.50)	58.14 58.02	4.88 4.64	12.33 12.19
17	169–170	74	$C_{32}H_{32}N_4O_{12}S$ (696.68)	55.17 54.92	4.63 4.52	8.04 7.85
18	188–189	76	$C_{24}H_{24}N_4O_8S$ (528.53)	45.54 45.42	4.58 4.51	10.60 10.47

Table 3. Spectral data of the newly synthesized compounds.

Compd.	IR (KBr, cm^{-1}), MS [m/z (%)], ^1H NMR [DMSO-d_6, δ, ppm]
2	IR: 1662 (C=O), 1730 (C=O), 3423 (NH). ^1H NMR: 1.22 (t, 3H, *J* = 5.6 Hz, C*H*$_3$CH$_2$), 4.21 (q, 2H, *J* = 5.6 Hz, CH$_3$C*H*$_2$), 4.57 (s, 2H, NC*H*$_2$), 7.23 (m, 4H, Ar-H), 7.30 (m, 3H, Ar-H), 7.39 (m, 3H, Ar-H), 9.92 (brs, 1H, NH). MS: 338 (M^+, 34).
3	IR: 1695 (C=O), 3427 (NH). ^1H NMR: 4.57 (s, 2H, NC*H*$_2$), 5.80 (brs, 2H, NH$_2$), 7.23 (m, 4H, Ar-H), 7.30 (m, 3H, Ar-H), 7.43 (m, 3H, Ar-H), 8.22 (brs, 1H, NH), 9.92 (brs, 1H, NH). MS: 324 (M^+, 25).
4	IR: 1692 (C=O), 3420 (NH). ^1H NMR: 2.43 (s, 6H, 2SCH$_3$), 4.44 (s, 2H, CH$_2$), 7.36 (m, 3H, Ar-H), 7.40 (m, 3H, Ar-H), 7.45 (m, 2H, Ar-H), 7.86 (m, 2H, Ar-H), 9.05 (bs, 1H, NH), 10.14 (bs, 1H, NH).
5a	IR: 1690 (C=O), 3350 (NH). ^1H NMR: 2.95 (t, 4H, *J* = 6.4 Hz, 2CH$_2$), 3.35 (t, 4H, *J* = 6.4 Hz, 2CH$_2$), 4.33 (s, 2H, CH$_2$), 7.37 (m, 4H, Ar-H), 7.31 (m, 3H, Ar-H), 7.42 (m, 3H, Ar-H), 8.01 (s, 1H, NH). MS: 415 [M^+ + H].
5b	IR: 1673 (C=O), 3341 (NH). ^1H NMR: 2.15 (m, 2H, CH$_2$), 2.24 (m, 4H, 2CH$_2$), 3.35 (t, 4H, *J* = 6.8 Hz, 2CH$_2$), 4.31 (s, 2H, CH$_2$), 7.33 (m, 4H, Ar-H), 7.37 (m, 3H, Ar-H), 7.44 (m, 3H, Ar-H), 7.97 (s, 1H, NH).
6a	IR: 1666 (C=O), 3349 (NH). ^1H NMR: 3.68 (s, 3H, OCH$_3$), 3.74 (s, 3H, OCH$_3$), 4.28 (s, 2H, CH$_2$), 7.06 (m, 4H, Ar-H), 7.31–7.34 (m, 4H, Ar-H), 7.44 (m, 3H, Ar-H), 7.61 (m, 2H, Ar-H), 7.71 (s, 1H, CH=N), 7.99 (s, 1H, NH), 9.72 (s, 1H, NH).
6b	IR: 1694 (C=O), 3405 (NH). ^1H NMR: 4.31 (s, 2H, CH$_2$), 7.05 (m, 4H, Ar-H), 7.11 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.37 (m, 2H, Ar-H), 7.49 (m, 2H, Ar-H), 7.67 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.70 (m, 2H, Ar-H), 7.75 (s, 1H, C*H*=N), 8.05 (s, 1H, NH), 9.80 (s, 1H, NH). MS: 446 [M^+].
6c	IR: 1695 (C=O), 3412 (NH). ^1H NMR: 3.72 (s, 6H, 2OCH$_3$), 3.84 (s, 3H, OCH$_3$), 4.28 (s, 2H, CH$_2$), 7.06 (m, 4H, Ar-H), 7.31–7.34 (m, 2H, Ar-H), 7.44 (m, 3H, Ar-H), 7.60 (m, 3H, Ar-H), 7.76 (s, 1H, C*H*=N), 7.99 (s, 1H, NH), 9.77 (s, 1H, NH).
7	IR: 1612 (C=N), 1692 (C=O), 3414 (NH). ^1H NMR: 4.83 (s, 2H, CH$_2$), 7.36 (m, 4H, Ar-H), 7.44 (m, 3H, Ar-H), 7.47 (m, 3H, Ar-H), 9.82 (s, 1H, NH), 12.52 (s, 1H, NH) **MS:** 360 [M^+].
8	IR: 1612 (C=N), 1692 (C=O), 3414 (NH). ^1H NMR: 2.52 (s, 3H, CH$_3$), 4.94 (s, 2H, CH$_2$), 7.35 (m, 4H, Ar-H), 7.40 (m, 3H, Ar-H), 7.45 (m, 3H, Ar-H), 9.8 (s, 1H, NH). MS: 380 [M^+].
9	IR: 3329 (NH), 3248 (NH$_2$), 1717(C=O), 1603 (C=O). ^1H NMR: 4.94 (s, 2H, CH$_2$), 5.82 (brs, 2H, NH$_2$), 7.32–7.36 (m, 4H, Ar-H), 7.44–7.47 (m, 3H, Ar-H), 7.52–7.55 (m, 3H, Ar-H), 9.82 (s, 1H, NH), 9.85 (s, 1H, NH).
10a	IR: 1611 (C=N), 1680 (C=O), 3321 (NH). ^1H NMR: 4.92 (s, 2H, CH$_2$), 7.31 (m, 4H, Ar-H), 7.42 (m, 3H, Ar-H), 7.48–7.52 (m, 5H, Ar-H), 7.71 (d, 2H, *J* = 7.5 Hz, Ar-H) 9.92 (s, 1H, NH), 10.12 (s, 1H, NH).
10b	IR: 1614 (C=N), 1672 (C=O), 3352 (NH). ^1H NMR: 4.94 (s, 2H, CH$_2$), 7.32 (m, 4H, Ar-H), 7.44 (m, 3H, Ar-H), 7.45-7.48 (m, 5H, Ar-H), 9.80 (s, 1H, NH), 9.85 (s, 1H, NH). MS: 471 [M^{+1}].
11a	IR: 3392 (OH), 1678 (CO), 1618 (C=N). ^1H NMR: 3.37 (m, 2H, H-5,5'), 3.39 (m, 1H, H-4), 3.56 (m, 1H, H-3), 4.22 (t, 1H, *J* = 6.4 Hz, OH), 4.87 (t, 1H, *J* = 6.2 Hz, OH), 4.95 (s, 2H, CH$_2$), 5.15 (t, 1H, *J* = 5.4 Hz, OH), 5.29 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-2), 5.39 (t, 1H, *J* = 6.6 Hz, OH), 7.12 (d, 1H, *J* = 9.8 Hz, H-1), 7.32–7.34 (m, 4H, Ar-H), 7.45–7.49 (m, 3H, Ar-H), 7.51 (m, 3H, Ar-H).

Table 3. cont.

Compd.	IR (KBr, cm^{-1}), MS [m/z (%)], ^1H NMR [DMSO-d_6, δ, ppm]
11b	IR: 3261 (OH), 1676 (CO), 1615 (C=N). ^1H NMR: 3.34 (m, 2H, H-6,6'), 3.39 (m, 1H, H-5), 3.54 (m, 2H, H-3,4), 4.19 (t, 1H, *J* = 6.4 Hz, OH), 4.39 (t, 1H, OH), 4.88 (t, 1H, *J* = 6.4 Hz, OH), 4.95 (s, 2H, CH_2), 5.14 (t, 1H, *J* = 5.8 Hz, OH), 5.24 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-2), 5.37 (t, 1H, *J* = 6.2 Hz, OH), 7.14 (d, 1H, *J* = 9.8 Hz, H-1), 7.30–7.34 (m, 4H, Ar-H), 7.42–7.47 (m, 3H, Ar-H), 7.50 (m, 3H, Ar-H)
12a	IR: 3453 (NH), 1750 (OAc), 1633 (CO). ^1H NMR: 1.97, 2.02, 2.05, 2.07 (4s, 12H, $4CH_3$), 3.97 (dd, 1H, *J* = 11.2 Hz, *J* = 2.8Hz, H-5'), 4.08 (dd, 1H, *J* = 11.2 Hz, *J* = 3.2 Hz, H-5''), 4.16 (m, 1H, H-4'), 4.62 (dd, 1H, *J* = 2.8 Hz, *J* = 6.5 Hz, H-3'), 5.27 (dd, 1H, *J* = 7.5.2 Hz, *J* = 9.2.5 Hz, H-2'), 5.66 (s, 2H, CH_2), 7.21 (d, 1H, *J* = 9.5 Hz, H-1'), 7.32–7.48 (m, 4H, Ar-H), 7.42–7.47 (m, 3H, Ar-H), 7.54 (m, 3H, Ar-H), 9.95 (s, 1H, NH).
12b	IR: 3428 (NH), 1748 (OAc), 1663 (CO). ^1H NMR: 1.93, 1.95, 2.03, 2.07, 2.10 (5s, 15H, $5CH_3$), 3.95 (dd, 1H, *J* = 11.2 Hz, *J* = 2.8Hz, H-6'), 4.12 (dd, 1H, *J* = 11.2 Hz, *J* = 3.2 Hz, H-6''), 4.18 (m, 1H, H-5'), 4.24 (t, 1H, *J* = 7.5 Hz, H-4'), 5.20 (dd, 1H, *J* = 2.8 Hz, *J* = 6.5 Hz, H-3'), 5.27 (dd, 1H, *J* = 7.5.2 Hz, J = 9.2.5 Hz, H-2'), 5.64 (s, 2H, CH_2), 7.22 (d,1H, J = 9.5 Hz, H-1'), 7.35–7.39 (m, 4H, Ar-H), 7.42–7.45 (m, 3H, Ar-H), 7.54 (m, 3H, Ar-H), 8.92 (s, 1H, NH).
13	IR: 3430 (NH), 1662 (CO). ^1H NMR: 3.42 (s, 3H, OCH_3), 4.14 (t, 2H, *J* = 5.8 Hz, CH_2), 4.93 (t, 2H, *J* = 5.8 Hz, CH_2), 4.98 (s, 2H, CH_2), 7.35–7.39 (m, 4H, Ar-H), 7.41–7.44 (m, 3H, Ar-H), 7.52 (m, 3H, Ar-H), 9.84 (s,1H, NH).
14	IR: 3386 (OH), 1664 (C=O). ^1H NMR: 3.82 (m, 2H, CH_2), 4.42 (d, 2H, CH_2), 4.69 (m, 1H, OH), 4.83 (s, 2H, CH_2), 4.90 (m, 1H, CH), 5.11 (m, 1H, OH), 7.38 (m, 4H, Ar-H), 7.49 (m, 3H, Ar-H), 7.48 (m, 3H, Ar-H), 10.42 (s, 1H, NH).
15	IR: 3419 (OH), 1656 (CO). ^1H NMR: 4.02 (t, 2H, *J* = 5.8 Hz, CH_2), 4.15 (t, 2H, *J* = 5.8 Hz, CH_2), 4.86 (m, 2H, CH_2), 4.92 (t, 2H, *J* = 5.8 Hz, CH_2), 505 (m, 1H, OH), 5.12 (s, 2H, CH_2), 7.37–7.40 (m, 4H, Ar-H), 7.47–7.55 (m, 3H, Ar-H), 7.66 (m, 3H, Ar-H), 9.93 (s,1H, NH).
17	IR: 3431(NH), 1747 (OAc), 1639 (CO). ^1H NMR: 1.89, 1.93, 2.02, 2.05, (4s, 12H, $4CH_3$), 3.90 (m, 1H, H-5), 4.05 (dd, 1H, $J_{6,6'}$ = 11.4 Hz, $J_{5,6}$ = 2.8 Hz, H-6), 4.16 (m, 1H, H-6'), 4.69 (t, 1H, *J* = 9.3 Hz, H-4), 4.73 (s, 2H, CH_2) 4.80 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.25 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.77 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 7.35–7.40 (m, 4H, Ar-H), 7.46–7.53 (m, 3H, Ar-H), 7.65 (m, 3H, Ar-H), 10.31 (s,1H, NH).
18	IR: 3388 (OH), 1665 (CO). ^1H NMR: 3.39 (m, 2H, H-6,6'), 3.46 (m, 1H, H-5), 3.59 (m, 2H, H-3,4), 4.26 (t, 1H, *J* = 6.4 Hz, OH), 4.48 (t, 1H, OH), 4.89 (t, 1H, OH), 4.92 (s, 2H, CH_2), 5.24 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-2), 5.35 (t, 1H, OH), 5.72 (d, 1H, *J* = 9.8 Hz, H-1), 7.41–7.49 (m, 4H, Ar-H), 7.52–7.57 (m, 3H, Ar-H), 7.72 (m, 3H, Ar-H).

12a,b. The ^1H NMR spectra of **12a** showed the signals of the *O*-acetylmethyl protons as singlet in the range δ 1.88–2.07 ppm, the rest of the sugar chain protons appeared in the range δ 3.94 – 5.49 ppm and the C-1 signal at δ 7.54 ppm (Scheme 2).

Reaction of thiole **7** with chloroethylmethyl ether, 2-(2-chloroethoxy)ethanol and 1-chloro-2,3-dihydroxypropane gave the corresponding *S*-substituted derivatives **13-15**, respectively. The ^1H NMR spectrum of **13** showed methyl signal at 3.42 ppm in addition to the CH_2 signals each as triplet. The IR spectra of compounds **14** and **15** revealed the presence of absorption bands of the hydroxyl groups at 3416–3486 cm^{-1}. Reaction of compound **7** with 2,3,4,6-tetra-*O*-acetyl-α-glucopyranosyl bromide (**16**) in acetone afforded the thioglycoside derivative **17**. Its IR spectrum showed the presence of absorption band at 1747 cm^{-1} corresponding to the *O*-acetyl carbonyl groups. Its ^1H NMR spectrum revealed the presence of the *O*-acetylmethyl groups at 1.89–2.05 ppm and the anomeric proton signal appeared at 5.77 ppm with coupling constant *J* = 9.8 Hz, indicating the ß-configuration of thioglucosidic linkage. The anomeric proton of ß-*N*-glucosides having an adjacent C=S, was reported to appear at higher chemical shift due to the anisotropic deshielding effect of the C=S. Deacetylation of thioglycoside **17** using methanolic ammonia solution at room temper-

ature afforded the deprotected thioglycoside **18** (Scheme 3).

Antimicrobial activity

The target compounds were screened *in vitro* for their antimicrobial activities against *Escherichia coli* NRRL B-210 (Gram negative bacteria), *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* (Gram positive bacteria), *Aspergillus niger* and *Candida albicans* NRRL Y-477 (fungi).

The results of the preliminary antimicrobial and the antifungal activities are shown in Table I. The results revealed that compounds **4, 9, 11a** and **14** showed varying degrees of inhibition against the previously mentioned bacteria. The best antifungal activity against *Aspergillus niger* was displayed by compounds **6**, **7**, **11a** and **12b**. Compounds **6, 7** and **11a** showed strong antifungal activity against *Candida albicans*. Some of the tested compounds showed relatively similar activities with inhibition zone values near to each other whereas other compounds showed little or no activity against one or more microorganisms.

Structure-activity relationship

The antimicrobial activity results and structure activity relationship indicated that the (oxadiazolylmethyl)phenytoin derivatives with attached acyclic arabinotetritolyl sugar moiety showed increased inhibition activities against both microorganism types. Furthermore, the hydrazinyl sugars with free hydroxyl groups showed higher activity that their corresponding acetylated analogs.

The antimicrobial activity results also proved that the attachment of hydrazinyl group to the substituted 1,3,4-oxadiazole system resulted in increased inhibition activity in relation to hydrazides of the phenytoin moiety. This is clear, as the activity increased in the oxadiazolyl hydrazine compared to the low activity of hydrazide **3**.

The acyclic nucleoside analogs with the oxadiazole ring system attached to acyclic hydroxyl oxygenated chain revealed higher inhibition activities against both microorganisms than the corresponding cyclic acetylated glucoside.

Additionally, free hydroxyl glucoside showed higher activity than its acetylated precursor. Furthermore, the dithiohydrazone and the free thiole-thione oxadiazole revealed higher inhibition activities than other synthesized mercapto derivatives.

The results revealed that the arylidine compound of the phenytoinhydrazide with phenyl ring carrying chlorine atom in the *para*-position showed higher activity that the corresponding analogs with methoxy groups.

CONCLUSION

New phenytoin derivatives, their oxadiazolyl, glycoside and some acyclic analogs were synthesized and evaluated for their antimicrobial activities. A number of the synthesized derivatives with sugar hydrazinyl and glucoside moieties as well as their acyclic analogs showed strong activities.

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