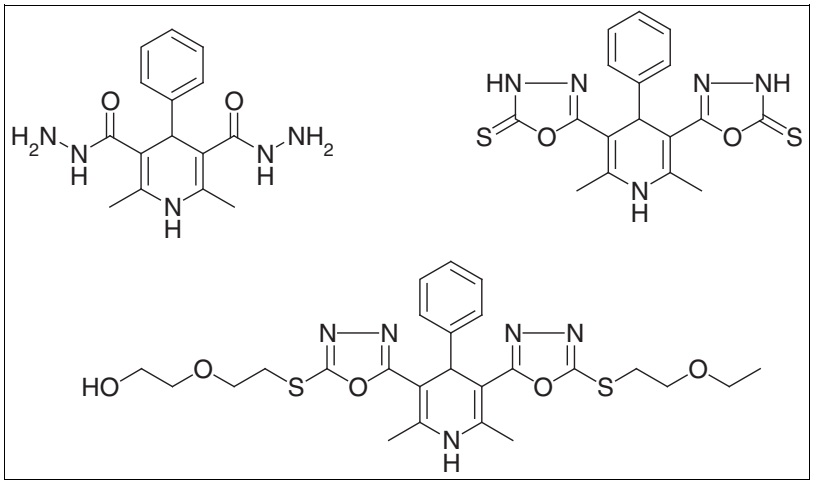
Utility of Hantzsch Ester in Synthesis of Some 3,5-Bis-dihydropyridine Derivatives and Studying Their Biological Evaluation

Eman R. Kotb,[a] Hebat-Allah S. Abbas,[a,b]* Eman M. Flefel,[a] Hayam H. Sayed,[a] and Nayera A. M. Abdelwahed[c]

[a]Photochemistry Department, National Research Centre, 12622 Dokki, Cairo, Egypt
[b]Chemistry Department, College of Science, King Khalid University, Abha 9004, Saudi Arabia
[c]Natural and Microbial Product Department, National Research Centre, 12622 Dokki, Cairo, Egypt
*E-mail: hebanrc@yahoo.com

Received June 26, 2012
DOI 10.1002/jhet.1848
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



The present work involves synthesis of new 3,5-bis-substituted dihydropyridine derivatives **2–16** starting from dihydropyridine-3,5-dicarboxylate (**1**) as starting material. Structures of new compounds were established by spectral and elemental analyses. Some of the new compounds were evaluated for anticancer and antimicrobial activity. Screening data of the tested compounds show promising anticancer and antimicrobial activity. The detail synthesis, spectroscopic data, and pharmacological activities are reported.

J. Heterocyclic Chem., **00,** 00 (2015).

INTRODUCTION

In our previous work, we found that pyridine derivatives showed a broad of biological activity such as antioxidant, antimicrobial, antitumor, and antiviral [1–7]. In addition, the literature reports support that pyridine derivatives are potent antitubercular [8] and anti-inflammatory agents [9]. Also, compounds containing 1,3,4-oxadiazole nucleuses are associated with diverse pharmacological activities, which have made them important chemotherapeutic agents [10,11], analgesic, diuretic, antihypertensive, anti-inflammatory [12], anticonvulsive [13], antibacterial, antifungal [14], and inhibit HIV replication [15]. In addition, some derivatives are active against hepatitis B and HIV-1 [16] viruses.

On the other hand, hydrazones containing azomethine (–NHN═CH) protons constitute a vital class of compounds for new drug development [17]. Several heterocyclic hydrazones were reported to possess various biological activities, antimicrobial [18], anti-inflammatory, analgesic [19], anticonvulsant [20], antitubercular, antiplatelet [21], antiviral, and antimalarial [22] activities. In view of these observations and in continuation of our work, we synthesized some heterocyclic compounds containing pyridine moiety and tested their biological activities.

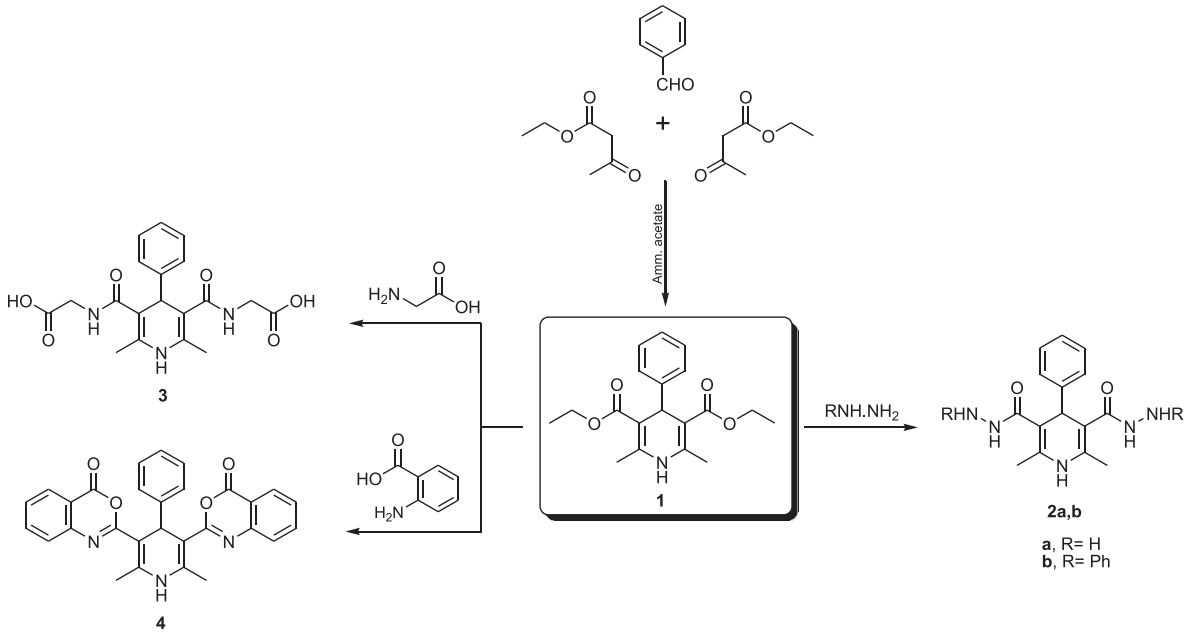
RESULT AND DISCUSSION

Chemistry. In the course of our investigation, we have found that diethyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**1**) [23] is an excellent building block for the synthesis of a numerous heterocyclic ring systems. The reactivity of 3,5-dicarboxylat **1** towards hydrazine derivatives was investigated. Thus, 3,5-dicarboxylate **1** was reacted with different nucleophiles namely hydrazine hydrate or phenyl hydrazine in ethanol, it afforded 1,4-dihydropyridine-3,5-dicarbohydrazide derivatives (**2a**,**b**) by hydrazinolysis method (Scheme 1). IR spectra of compound **2a** revealed absorption bands for NH_2, NH, and (C═O), and its mass spectrum showed a peak corresponding to its molecular ion peak at *m*/*z* (%) = 301 (35) [M^+] (cf. Experimental).

Further reaction of compound **1** with different amino acids, namely glycine and anthranilic acid, it afforded the corresponding compounds **3**, **4** respectively (Scheme 1). IR spectra of compound **3** revealed absorption bands for OH, NH, and (C═O), and its ^{1}H NMR spectrum showed the presence of OH protons, NH, and (CH_2) signals (cf. Experimental).

1,4-Dihydropyridine-3,5-dicarbohydrazide **2a** was proved chemically via condensation with different acid anhydride

Scheme 1

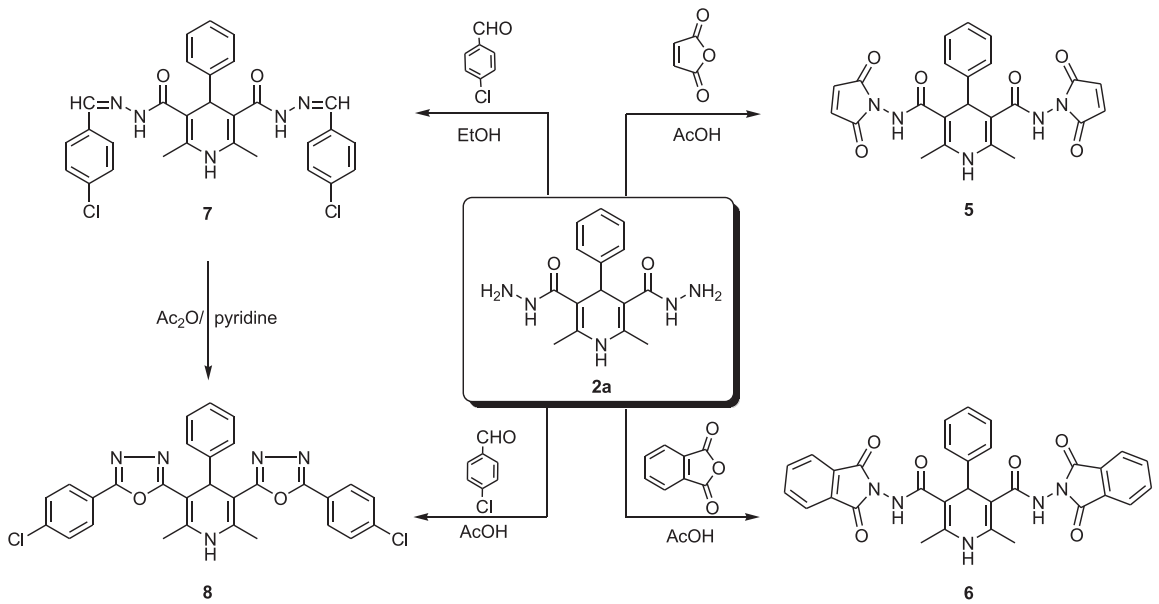


namely, maleic anhydride and phthalic anhydride in acetic acid gave the corresponding *N*-amide derivatives **5**, **6** respectively (Scheme 2). All the prepared compounds were provided via elemental analysis and spectral data (cf. Experimental).

Also, the treatment of hydrazide **2a** with *p*-chlorobenzaldehyde in ethanol afforded the corresponding Schiff's base compound **7** on the basis of its spectral data (Scheme 2). The IR spectrum showed the disappearance of the presence of (NH_2) stretching vibration in the spectrum of hydrazide **2a**, and their ^{1}H NMR spectra showed the presence of NH and azo-methine (CH═N) signals (cf. Experimental).

Bis(4-chlorobenzylidine)-1,4-dihydropyridine-3,5-dicarbohydrazide (**7**) was cyclized into the corresponding 1,4-dihydropyridine-bis-1,3,4-oxadiazole derivative **8** via refluxing in a mixture of acetic anhydride/pyridine (2:1). The IR spectrum of **8** showed absence of the bands

Scheme 2



corresponding to (C═O) group. Also, the structure of compound **8** was established chemically upon condensation of **2a** with *p*-chlorobenzaldehyde in acetic acid (cf. Experimental).

The bis-hydrazone derivative **9** (Scheme 3) was prepared via reaction of compound **2a** with monosaccharide: namely, D-glucose in the presence of catalytic amounts of glacial acetic acid. The products revealed absorption bands for OH, NH, (C═O), and (C═N) in IR spectra, and their ^{1}H NMR spectra showed the presence of the sugar protons, NH, and azo-methine (CH═N) signals (cf. Experimental).

Acetylation of bis-hydrazone **9** with acetic anhydride at room temperature gave the bis-*O*-acetylated sugar derivative **10**. The IR spectrum of latter compound revealed the absence of hydroxyl group. The ^{1}H NMR spectrum showed the absence of OH groups and the presence of OAc groups (cf. Experimental). Oxidative cyclization of compounds **10** using bromine/acetic acid [24,25] afforded the corresponding bis-*O*-acetylated cyclic *C*-nucleoside of 1,3,4-oxadiazoline derivative **11** (Scheme 3). The IR spectrum of compound **11** showed absorption bands corresponding to (O–C═O) and (N–C═O) groups. The ^{1}H NMR spectrum showed the absence of azo-methine (CH═N) and the presence of *O*-acetyl-methyl protons at δ 2.08–2.25 ppm and *N*-acetyl-methyl protons at 2.41 ppm (cf. Experimental).

Deprotection of **11** using ammonium hydroxide solution in methanol, [26] gave the target free cyclic *C*-nucleoside **12** (Scheme 3). The structure of the aforementioned compound was confirmed on the basis of their spectral data. The IR spectra revealed absorption bands due to (OH) and (C═N); whereas their ^{1}H NMR spectra showed signals of the alditol protons congregated with the solvent absorption [40] and the presence of hydroxyl groups (exchangeable with D_2O) (cf. Experimental).

Also, compound **2a** was treated with CS_2/KOH to give 1,4-dihydropyridine-bis-1,3,4-oxadizole thione derivative **13** (Scheme 4). In the IR spectrum of compound **13**, no signal derived from exocyclic carbonyl function was observed. Moreover, $NHNH_2$ stretching vibration was disappeared.

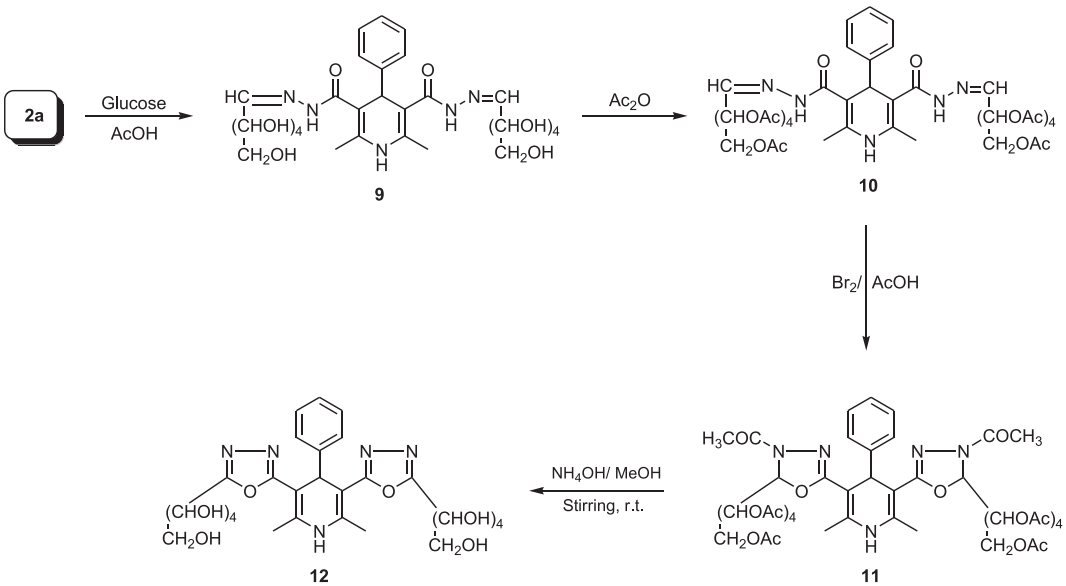
Because of the pharmacological activities of acyclovir [27], many attempts have been made by nucleoside chemists to prepare a number of related compounds with various side chains and glycons [27]. Thus, when the sodium salt of compound **13** (generated *in situ*) was treated with 2-(2-chloroethoxy) ethanol, epichlorohydrine, and 2-chloro ethanol, it afforded the corresponding *S*-acyclic nucleosides derivatives **14–16**, respectively.

The structure of the aforementioned acyclic nucleosides was confirmed with spectral data, and ^{1}H NMR spectra revealed hydroxylethoxy ethyl, oxiran ring, and hydroxyethyl signals. In addition, the IR, ^{13}C NMR spectra revealed that the side of attack was on the *S*-atom (cf. Experimental).

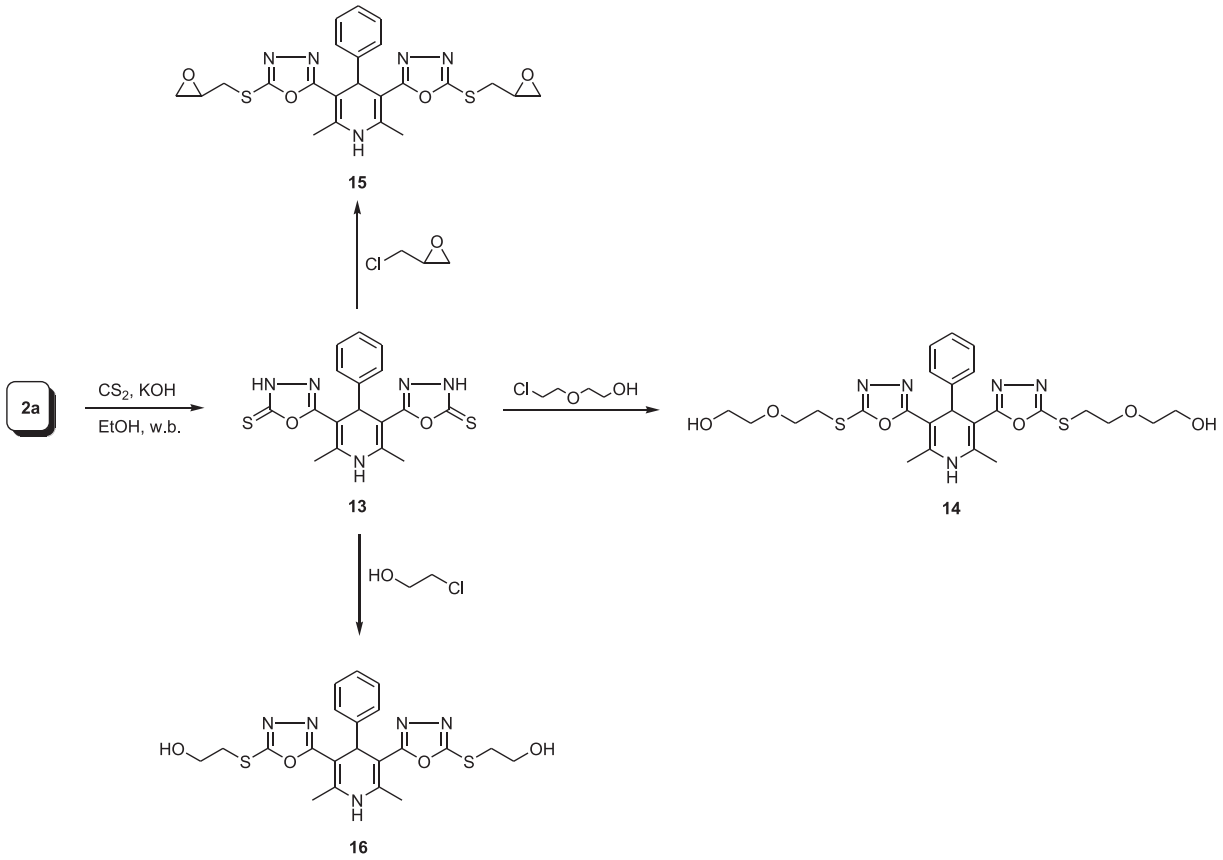
Biological evaluation

Anticancer activity. Chemotherapy is a major therapeutic approach for both localized and metastasized cancers. In the present work, nine of the newly synthesized compounds **2a**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and **16** were selected to evaluate their *in vitro* growth inhibitory activities against human cancer cell line, which is breast cell line (T47D) in comparison with the known anticancer drug, doxorubicin (DOX) as a reference drug. The anticancer activity results indicated that most of the synthesized compounds showed

Scheme 3



Scheme 4



anticancer activity against breast cell line (T47D) but with varying intensities in comparison with DOX. Moreover, compounds **14** and **16** showed the good cytotoxic activity IC_{50} 22 µg/mL (Table 1), compounds **6** and **15** showed moderate cytotoxic activity IC_{50} 23 µg/mL, and compounds **5**, **9**, and **10** showed cytotoxic activity IC_{50} 24–25 µg/mL but compounds **2a** and **4** showed no cytotoxic activity.

Table 1

Effect of some selected newly synthesized compounds on breast cancer cell line (T47D).

Comp.	IC_{50}
2a	==
4	==
5	24 µg mL
6	23 µg mL
9	25 µg mL
10	25 µg mL
14	22 µg mL
15	23 µg mL
16	22 µg/mL
DOX	7.8 µg/mL

IC_{50}: dose of the compounds that reduces survival to 50%.

The variety of antitumor activities might be explained because of the difference of side chains. The highest activity of compounds **14** and **16** was explained because of the presence of *S*-acyclic sugar derivatives.

Results were illustrated in Figure 1 for the cytotoxic activities of the compounds (**2a**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and **16**) in comparison with DOX.

Antimicrobial activity. As shown in Table 2, the antimicrobial effect of the tested compounds was evaluated by measuring the zone diameters and their results were compared with those of well-known drugs (standards). It

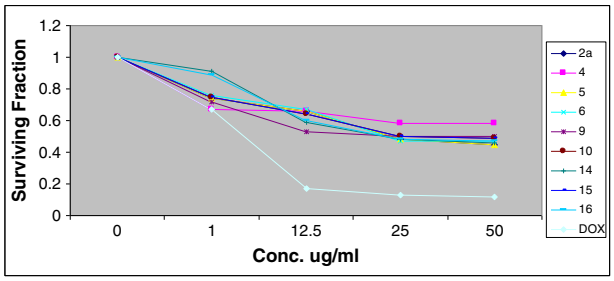


Figure 1. Cytotoxic effect of compounds **2a**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and **16** on breast cell line (T47D) to (DOX). [Color figure can be viewed in the online issue, which is available at http://www.interscience.wiley.com.]

Table 2

Inhibition zones of the newly synthesized compounds.

	Inhibition zone				
	Gram-positive bacteria		Gram-negative bacteria	Fungi	Yeast
Compd. no.	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillus niger*	*Candida albicans*
DMSO (solvent)	–	–	–	–	–
2a	++	–	–	–	–
2b	++	–	–	+	+
5	+++	+++	–	++	+++
6	++	+	–	+	+
7	+++	+++	–	++	+++
9	++	+	–	+	+
10	++	+	–	+	+
12	++++	++++	++	+++	++++
13	+++	+++	–	++	+++
14	++	+	–	+	+
15	++	+	–	+	+
16	+++	+++	–	++	+++
Ciprofloxacin ($50\,\mu g\,mL^{-1}$)	++++	++++	++++	–	–
Ketaconazole ($50\,\mu g\,mL^{-1}$)	–	–	–	++++	++++

–, no antimicrobial effect.
+, low antimicrobial effect (4 mm).
++, moderate antimicrobial effect (8–10 mm).
+++, high antimicrobial effect (15–18 mm).
++++, complete antimicrobial effect (20–22 mm).

Table 3

MIC of the newly synthesized compounds.

	MIC (mg/mL^{-1})				
	Gram positive bacteria		Gram negative bacteria	Fungi	Yeast
Compd. no.	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillus Niger*	*Candida albicans*
DMSO (solvent)	–	–	–	–	–
2a	0.45	–	–	–	–
2b	0.45	–	–	0.65	0.65
5	0.25	0.25	–	0.45	0.25
6	0.45	0.65	–	0.65	0.65
7	0.45	0.25	–	0.45	0.25
9	0.25	0.45	–	0.65	0.65
10	0.45	0.65	–	0.65	0.65
12	0.075	0.075	0.45	0.25	0.075
13	0.25	0.25	–	0.45	0.25
14	0.45	0.65	–	0.65	0.65
15	0.45	0.65	–	0.65	0.65
16	0.25	0.25	–	0.45	0.25

MIC, minimum inhibitory concentration.

is evident that most tested compounds display activity against *Bacillus subtilis* and *Staphylococcus aureus* but only compound **12** showed moderate activities against *Escherichia coli*. While all the tested compounds except **2a**, **2b** were active against *Aspergillus Niger* and *Candida albicans*, compound **12** was the most active one against all the listed bacteria, fungi, and yeast. Also, compounds **5**, **7**, **13**, and **16** showed significant antimicrobial activity. The

minimal inhibitory concentrations (MIC) of these compounds ranged from 0.075 to 0.65 mg mL^{-1} (Table 3).

CONCLUSION

In the present work, 16 new 3,5-bis-dihydropyrisine derivatives were synthesized and characterized using spectral and elemental analyses. Some of the synthesized compounds evaluate their *in vitro* growth inhibitory activities against human cancer breast (T47D) cell line and showed good activity because of the presence of *S*-acyclic nucleoside derivatives.

Screening data of the prepared compounds show promising antibacterial and antifungal activities. Compounds **12** showed more significant antibacterial activity because of the presence of the free cyclic *C*-nucleoside than compounds **5**, **7**, **13**, and **16**.

EXPERIMENTAL

Chemistry. All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, Shimadzu (Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer, National Research Centre, Cairo, Egypt. NMR spectra were determined on a Jeol-Ex-300 (^{1}H NMR, ^{13}C NMR) spectrometer, and chemical shifts were expressed as parts per million (ppm) (δ values) against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on EI + Q1 MSLMR UPLR, (Faculty of Science, Cairo University, Cairo, Egypt). Antitumor screening made by the National Cancer Institute, Cairo University, Cancer Biology Department, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-coated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany).

Synthesis of 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2a). A mixture of compound **1** (3.29 g, 1 mmol) and hydrazine hydrate 99% (3 mL) in absolute ethanol (30 mL) was refluxed for 8 h. After cooling, the reaction mixture was poured into cold water, the formed solid was filtered off and recrystallized from dioxane to give compound **2a** as white powder, mp 137–139°C; 1.5 g (50%); IR (KBr) ν: 3398–3350 ($2NH_2$), 3165, 3148 (3NH), 1678 (2C═O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.1 (s, 4H, $2NH_2$, exchangeable with D_2O), 2.36 (s, 6H, $2CH_3$), 4.94 (s, 1H, pyridine-H), 7.0–7.2 (m, 5H, Ar–H), 8.1 (s, 2H, 2NH, exchangeable with D_2O), 9.2 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 14.8, 17.9 ($2CH_3$), 49.2 (CH pyridine), 103.2, 104.6, 122.2, 125.8, 126.2, 129.5, 147.4, 149.5, 150.3 (10 C–Ar), 165.15 (2C═O). MS: *m/z* (%) = 301 (35) [M^+]. *Anal*. Calcd for $C_{15}H_{19}N_5O_2$ (301.34): C, 59.79; H, 6.36; N, 23.24. Found: C, 59.90; H, 6.28; N, 23.35.

Synthesis of 2,6-dimethyl-N^3,N^5,4-triphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2b). A mixture of compound **1** (3.29 g, 1 mmol) and phenyl hydrazine (2.16 g, 2 mmol) in 30 mL absolute ethanol was refluxed for 6 h. After cooling, the separated solid was recrystallized from ethanol to give compound **2b** as pale reddish crystals, mp 124–126°C; 3.62 g (80%); IR (KBr) ν: 3210, 3160, 3128 (5NH), 1674 (2C═O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 6H, $2CH_3$), 2.41 (s, 2H, 2NH, exchangeable with D_2O), 4.99 (s, 1H, pyridine-H), 6.5–7.3 (m, 15H, Ar–H), 8.06 (s, 2H, 2NH, exchangeable with D_2O), 9.08 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 14.1, 16.6 ($2CH_3$), 50.3 (CH pyridine), 103.6, 104.9, 113.3, 113.9, 121.5, 125.3, 125.9, 129.15, 147.4, 149.5, 150.3, 155.2 (22 C-Ar), 164.9, 165.07 (2C═O). MS: *m/z* (%) = 453 (29) [M^+]. *Anal.* Calcd for $C_{27}H_{27}N_5O_2$ (453.54): C, 71.5; H, 6.0; N, 15.44. Found: C, 71.41; H, 6.10; N, 15.52.

Synthesis of 2,2′[2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)bis(carbonylimino)]diacetic acid (3). Glycine (0.15 g, 2 mmol) and Na_2CO_3 (15 mmol) were dissolved in water (15 mL), and the pH was adjusted to 9–9.5. Then the compound **1** (3.29 g, 1 mmol) dissolved in ethanol (20 mL) was added, and the reaction mixture was stirred at 100°C for 8 h at the controlled pH. The reaction mixture was left overnight at room temperature then treated with cold formic acid. The solid obtained was filtered off, washed with H_2O, and crystallized from methanol to yield the corresponding compound **3** as pale white crystals, mp 158–159°C; 1.89 g (49%); IR (KBr) ν: 3412–3341, 3239, 3160 (2OH, 3NH), 1689, 1671, 1650 (4C═O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 6H, $2CH_3$), 2.42 (s, 2H, 2NH, exchangeable with D_2O), 3.91 (s, 4H, $2CH_2$), 5.01 (s, 1H, pyridine-H), 6.89–7.01 (m, 5H, Ar–H), 9.1 (s, 1H, NH pyridine, exchangeable with D_2O), 11.05 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 13.9, 14.2 ($2CH_3$), 43.2, 43.9 ($2CH_2$), 48.6 (CH pyridine), 102.4, 103.2, 121.5, 126.3, 126.9, 129.5, 147.4, 149.5 (10 C–Ar), 165.02, 165.12, 171.3, 171.9 (4C═O). MS: *m/z* (%) = 387 (12) [M^+]. *Anal*. Calcd for $C_{19}H_{21}N_3O_6$ (387.39): C, 58.91; H, 5.46; N, 10.85. Found: C, 59.01; H, 5.38; N, 10.93.

Synthesis of 2,2′(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)bis-4H-3,1-benzoxazin-4-one (4). Anthranilic acid (2.8 g, 2 mmol) and compound **1** (3.29 g, 1 mmol) were fused in conical flask at 110°C on oil bath for 15 h. The reaction mixture was cooled and the formed precipitate was filtered off and recrystallized from methanol to yield the compound **4** as pale brown powders, mp 182–183°C; 1.99 g (42%). IR (KBr) ν: 3239 (NH), 1689, 1679 (2C═O), 1605 (C═N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6H, $2CH_3$), 5.06 (s, 1H, pyridine-H), 6.76–7.32 (m, 13H, Ar–H), 9.08 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 16.1, 16.5 ($2CH_3$), 44.6 (CH pyridine), 99.9, 102.8, 103.5, 112.6, 121.6, 122.4, 123.6, 141.1, 142.1, 142.9, 143.4, 150.6, 155.2, 156.4 (24 C–Ar), 161.3, 161.8 (2C═O). MS: *m/z* (%) = 475 (16) [M^+]. *Anal.* Calcd for $C_{29}H_{21}N_3O_4$ (475.49): C, 73.25; H, 4.45; N, 8.84. Found: C, 73.32; H, 4.51; N, 8.74.

Synthesis of compounds (5, 6). ***General procedure.*** A mixture of compound **2a** (3.01 g, 1 mmol) and maleic anhydride or phthalic anhydride (2 mmol) was refluxed in glacial acetic acid (30 mL) for 6 h. The reaction mixture was cooled and poured into ice/water; the solid that formed was filtered off, dried, and recrystallized from n-butanol.

Synthesis of N^3,N^5-bis(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxamide (5). As white crystals, mp 89–91°C; 2.58 g (56%); IR (KBr) ν 3200, 3190, 3160 (3NH), 1710, 1703, 1685, 1676 (6C═O) cm^{-1}). ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H, $2CH_3$), 4.93 (s, 1H, pyridine-H), 6.92 (d, 4H, pyrrole ring), 7.06–7.23 (m, 5H, Ar–H), 8.10 (s, 2H, 2NH amide, exchangeable with D_2O), 9.15 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 17.6 ($2CH_3$), 49.1 (CH pyridine), 102.5,

104.9, 113.4, 113.9, 1221.3, 123.4, 124.7, 143.2, 144.7, 147.9 (14C-Ar), 163.6, 164.1, 165.3, 165.9 (6C=O). MS: *m/z* (%) = 461 (27) [M^+]. *Anal*. Calcd for $C_{23}H_{19}N_5O_6$ (461.43): C, 59.87; H, 4.15; N, 15.18; Found: C, 59.92; H, 4.08; N, 15.09.

Synthesis of N^3,N^5-bis(1,3-dioxoisoindolin-2-yl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxamide (6). As brown crystals, mp 261–262°C; 4.65 (83%). IR (KBr) ν: 3195, 3182, 3151 (3NH), 1709, 1705, 1679, 1669 (6C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.31 (s, 6H, $2CH_3$), 5.03 (s, 1H, pyridine-H), 7.09–7.21 (m, 5H, Ar–H), 7.72–8.06 (m, 10H, Ar–H + 2NH, exchangeable with D_2O), 9.23 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 15.9, 16.3 ($2CH_3$), 48.2 (CH pyridine), 103.9, 104.1, 121.3, 125.4, 126.2, 129.5, 132.5, 133.6, 133.9, 134.2, 144.5, 145.8 147.4, 149.5, 150.3 (22 C–Ar), 164.2, 164.8, 165.09, 165.17, 165.78 (6C=O). MS: *m/z* (%) = 561 (15) [M^+]. *Anal*. Calcd for $C_{31}H_{23}N_5O_6$ (561.54): C, 66.30; H, 4.13; N, 12.47; Found: C, 66.39; H, 4.23; N, 12.55.

Synthesis of N^3,N^5-bis(4-chlorobenzylidene)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (7). To a mixture of compound **2a** (3.01 g, 1 mmol) in 30 mL ethanol in the presence of 0.1 mL piperidine, *p*-chlorobenzaldehyde (2.82 g, 2 mmol) was added. The reaction mixture was refluxed for 3 h, the formed solid was filtered off, dried, and recrystallized from acetic acid to give **7** as pale brown powders, mp 181–182°C, 5.01 g (92%). IR (KBr) ν: 3183, 3148, 3129 (3NH), 1668, 1661 (2C=O), 1608, 1602 (2C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.29 (s, 6H, $2CH_3$), 4.96 (s, 1H, pyridine-H), 7.11–7.45 (m, 13H, Ar–H), 8.0 (s, 2H, 2NH, exchangeable with D_2O), 8.2 (s, 2H, azomethine proton), 9.06 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 19.6 ($2CH_3$), 48.3 (CH pyridine), 104.2, 104.9, 111.9, 113.2, 114.09, 121.8, 122.4, 124.8, 126.95, 130.3, 132.9, 133.04, 144.3, 145.1, 145.33 (22 C–Ar), 148.5 (2C=N), 165.3, 165.9 (2C=O). MS: *m/z* (%) = 545 (27) [M^+], 547 (14) [M^++2]. *Anal*. Calcd for $C_{29}H_{25}Cl_2N_5O_2$ (546.45): C, 63.74; H, 4.61; N, 12.82; Found: C, 63.68; H, 4.69; N, 12.99.

Synthesis of 5,5′-(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)bis-(2-(4-chlorophenyl)-1,3,4-oxadiazol) (8). ***Method A.*** A solution of compound **7** (5.45 g, 1 mmol) in 30 mL of acetic anhydride/pyridine (2:1) was refluxed for 2 h. The reaction mixture poured onto water (100 mL), the formed solid was filtered off, dried, and recrystallized from acetic acid to give **8**.

Method B. To a solution of compound **2a** (3.01 g, 1 mmol) in 30 mL glacial acetic acid, *p*-chlorobenzaldehyde (2.82 g, 2 mmol) was added. The reaction mixture was refluxed for 3 h, the formed solid was filtered off, dried, and recrystallized from acetic acid to give **8** as brown crystals, mp 211–212°C; 3.90 g (72%). IR (KBr) ν: 3123 (NH), 1608, 1601 (4C=N) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.33 (s, 6H, $2CH_3$), 4.99 (s, 1H, pyridine-H), 7.01–7.43 (m, 13H, Ar–H), 9.16 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 16.1, 16.86 ($2CH_3$), 48.06 (CH pyridine), 103.2, 104.6, 111.26, 112.08, 121.03, 121.98, 122.2, 125.8, 126.2, 129.5, 133.04, 133.49, 141.64, 144.19, 145.32 (22 C–Ar), 149.21, 149.59 (4C=N). MS: *m/z* (%) = 541 (40) [M^+], 543 (15) [M^++2]. *Anal*. Calcd for $C_{29}H_{21}Cl_2N_5O_2$ (542.42): C, 64.21; H, 3.90; N, 12.91; Found: C, 64.11; H, 3.99; N, 12.79.

Synthesis of 2,6-dimethyl-N^3,N^5-bis(2,3,4,5,6-pentahydroxy-hexylidene)-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (9). A mixture of compound **2a** (3.01 g, 1 mmol) and D-glucose (3.6 g, 2 mmol) in 50 mL ethanol in the presence of 1 mL acetic acid as catalyst was heated at 70°C for 2 h. The formed precipitate was filtered off, washed with water, dried, and recrystallized from ethanol to give **9** pale white crystals, mp 177–179°C; 4.06 g (65%). IR (KBr) ν: 3435–3220 (10 OH + 3NH), 1673, 1668 (2C=O), 1603 (2C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.31 (s, 6H, $2CH_3$), 3.1–3.34 (m, 4H, $2CH_2OH$), 3.39–4.05 (m, 8H, alditol proton), 4.87–5.02 (m, 6H, pyridine-H + 5OH, exchangeable with D_2O), 5.3–5.74 (m, 5H, 5OH, exchangeable with D_2O), 7.08–7.19 (m, 5H, Ar–H), 8.05 (s, 2H, 2NH, exchangeable with D_2O), 8.3 (s, 2H, 2 N=CH), 9.16 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 15.3, 16.01 ($2CH_3$), 28.23, 28.93 ($2CH_2$), 44.09 (CH pyridine), 61.21, 62.36, 62.98, 71.32, 72.54, 73.15, 73.89 (8 C-alditol), 103.2, 104.6, 122.2, 125.8, 126.2, 129.5, 147.4, 149.5, 150.3 (10 C–Ar), 158,21, 159.01 (2C=N), 165.56 (2C=O). MS: *m/z* (%) = 625 (19) [M^+]. *Anal*. Calcd for $C_{27}H_{39}N_5O_{12}$ (625.62): C, 51.83; H, 6.28; N, 11.90; Found: C, 51.75; H, 6.32; N, 12.05.

Synthesis of 2,6-dimethyl-N^3,N^5-bis(2,3,4,5,6-penta-acetoxcy-hexylidene)-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (10). A solution of compound **9** (6.25 g, 1 mmol) in acetic anhydride 30 mL was stirred at room temperature over night. The reaction mixture was poured into crushed ice; the precipitate solid was filtered off, dried, and recrystallized from ethanol to give **10** as pale white crystals, mp 281–283°C; 5.85 g (56%). IR (KBr) ν: 3140, 3127, 3122 (3NH), 1755, 1750, 1742, 1673, 1668 (12C=O), 1607, 1601 (2C=N) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.09–2.28 (m, 30H, 10 $COCH_3$), 2.43 (s, 6H, $2CH_3$), 3.23–3.37 (m, 4H, $2CH_2OAc$), 4.41–4.81 (m, 4H, 4*CH*OAc), 5.19–5.23 (m, 5H, pyridine-H + 4*CH*OAc), 7.18–7.24 (m, 7H, Ar–H + 2NH, exchangeable with D_2O), 8.3 (s, 2H, 2N=CH), 9.32 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 17.21–19.26 ($12CH_3$), 24.32, 24.50 ($2CH_2$), 44.59 (CH pyridine), 61.21, 62.51, 63.02, 71.48, 72.16, 73.15, 73.94 (8 C-alditol), 103.2, 104.6, 109.26, 121.68, 122.78, 124.26, 126.2, 129.5, 147.4, 149.5 (10 C–Ar), 158,78, 159.11 (2C=N), 165.06–166.89 (12C=O). *Anal*. Calcd for $C_{47}H_{59}N_5O_{22}$ (1045.99): C, 53.97; H, 5.69; N, 6.70; Found: C, 54.01; H, 5.80; N, 6.61.

Synthesis of 1,1′-(5,5′-(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)-bis[2-(2,3,4,5,6-pentaacetoxcyhexylidene)-1,3,4-oxadiazole-5,3-(2H)-diyl]diethanone (11). A solution of compound **10** (1.45 g, 1 mmol) in mixture of glacial acetic acid/bromine (3:1) 30 mL was stirred at room temperature over night. The reaction mixture was poured into crushed ice; the separated solid was filtered off, dried, and recrystallized from ethanol to give **11** as pale reddish crystals, mp over 300°C; 4.86 g (43%). IR (KBr) ν: 3135 (NH), 1753, 1742, 1750, 1740, 1679, 1671 (10C=O), 1669 (N–C=O), 1606, 1601 (2C=N) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.08–2.25 (m, 30H, 10 $COCH_3$), 2.34 (s, 6H, $2CH_3$), 2.47 (m, 6H, 2N–$COCH_3$), 3.21–3.35 (m, 4H, $2CH_2OAc$), 4.48–4.69 (m, 4H, 4*CH*OAc), 5.07–5.26 (m, 4H, pyridine-H + 4*CH*OAc), 5.49 (s, 2H, 2 oxadiazole-H), 7.18–7.24 (m, 5H, Ar–H), 9.27 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 16.29–20.58 ($14CH_3$), 24.12, 24.45 ($2CH_2$), 45.26 (CH pyridine), 61.89, 62.26, 63.65, 72.03, 72.86, 73.82, 74.14 (8 C-alditol), 103.5, 104.32, 108.56, 121.68, 122.01, 123.27, 126.89, 129.35, 144.4, 147.26 (12C–Ar), 159.23, 159.98 (2C=N), 164.26–166.93 (12C=O). *Anal*. Calcd for $C_{51}H_{63}N_5O_{24}$ (1130.07): C, 54.20; H, 5.62; N, 6.20; Found: C, 54.11; H, 5.74; N, 6.31.

Synthesis of 1,1′-(5,5′-(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)-bis[2-(2,3,4,5,6-pentahexylidene)-1,3,4-oxadiazole (12). To a solution of compound **11** (11.3 g, 1 mmol) in anhydrous methanol (50 mL), ammonium hydroxide solution (5 mL, 35%) was added, then the reaction mixtures were stirred at room temperature for 3 h. The reaction mixtures were evaporated under reduced pressure at 40°C, and the residues were purified on silica gel column using chloroform : methanol (4:1) as an eluent to give products as brown crystals, mp over 288–289°C; 1.42 g (23%). IR (KBr) ν: 3425–3258, 3135 (10OH, NH), 1606, 1601 (4C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 6H, $2CH_3$), 3.08–3.29 (m, 4H, $2CH_2$OH), 3.31–4.10 (m, 8H, alditol proton), 4.86–4.93 (m, 5H, 5OH), 5.09–5.26 (m, 6H, pyridine-H + 5OH), 7.05–7.19 (m, 5H, Ar–H), 9.03 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) (δ, ppm): 17.21, 17.83 ($2CH_3$), 23.02, 23.19 ($2CH_2$), 44.48 (CH pyridine), 61.21, 62.89, 63.24, 71.08, 71.66, 72.35, 73.34 (8 C-alditol), 103.29, 104.32, 109.06, 111.05, 121.68, 122.78, 124.26, 126.32, 129.59, 147.14, 149.09 (10 C–Ar), 159.01, 159.65, 160.82, 161.05 (4C=N). MS: *m/z* (%) = 621 (27) [M^+]. *Anal.* Calcd for $C_{27}H_{35}N_5O_{12}$ (621.59): C, 52.17; H, 5.68; N, 11.27; Found: C, 52.23; H, 5.79; N, 11.16.

Synthesis of 5,5′-(2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-diyl)bis-(1,3,4-oxadiazole-2(3H)-thione) (13). To a warmed solution of KOH (1.12 g, 2 mmol in 2 mL water: 30 mL ethanol), compound **2a** (3.01 g, 1 mmol) was added. The reaction mixture was heated for 15 min, and the mixture was cold to room temperature, and 2 mL carbon disulfide was added. The reaction mixture was heated under reflux for 10 h, and then poured into crushed ice. The formed solid was filtrated off and recrystallized from ethanol to give **13** as brown crystals, mp 161–162°C; 2.46 g (64%). IR (KBr) ν: 3145, 3138, 3129 (3NH),1610, 1604 (2C=N) 1228, 1220 (2C=S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H, $2CH_3$), 5.06 (s, 1H, pyridine-H), 7.08–7.27 (m, 7H, Ar–H + 2NH, exchangeable with D_2O), 9.09 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 17.8 ($2CH_3$), 45.6 (CH pyridine), 103.4, 104.7, 123.2–143.6 (10 C–Ar), 155.1 (2C=N), 157.6, 158.2 (2C=S). MS: *m/z* (%) = 385 (20) [M^+]. *Anal.* Calcd for $C_{17}H_{15}N_5O_2S_2$ (385.46): C, 52.97; H, 3.92; N, 18.17; S, 16.64; Found: C, 53.07; H, 3.82; N, 18.23; S, 16.73.

Alkylation of bis 1,3,4-oxadiazole thione derivatives. ***Synthesis of compounds (14–16): General procedure.*** To a solution of **13** (3.85 g, 1 mmol) in 20 mL DMF, sodium hydride (0.48 g, 2 mmol) was added. The reaction mixture was stirred at 60°C for 1 h, then the reaction mixture was cooled then added 2-(2-chloroethoxy) ethanol, epichlorohydrine or 2-chloro ethanol (2 mmol) was added then stirred at room temperature over night. The reaction mixture was evaporated under reduced pressure; the residue was washed with distilled water, filtered off, dried, and recrystallized from methanol.

Synthesis of 3,5-bis-(2(2-hydroxyethoxy)ethylthio)-1,3,4-oxzadiazol-2-yl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridin (14). As white crystals, mp 141–143°C; 2.69 g (48%). IR (KBr) ν: 3352 (2OH), 3122 (NH), 1610, 1605 (4C=N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.1 (s, 2H, 2OH, exchangeable with D_2O), 2.29 (s, 6H, $2CH_3$), 3.1 (t, 4H, $2CH_2$, J = 6.1 Hz), 3.41 (t, 4H, $2CH_2$, J = 4.2 Hz), 3.64 (t, 4H, $2CH_2$, J = 6.2 Hz), 3.74 (t, 4H, $2CH_2$, J = 4.2 Hz), 4.99 (s, 1H, pyridine-H), 7.01–7.16 (m, 5H, Ar–H), 9.32 (s, 1H, NH pyridine, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 16.32, 16.87 ($2CH_3$), 45.65 (CH pyridine), 46.59–63.2 (8 CH_2), 103.87, 105.06, 111.26, 122.68, 122.98, 124.06, 126.32, 129.5, 147.4 (10 C–Ar), 155.26, 156.72, 158,78, 159.11 (4C=N). MS: *m/z* (%) = 561 (38) [M^+]. *Anal.* Calcd for $C_{25}H_{31}N_5O_6S_2$ (561.67): C, 53.46; H, 5.56; N, 12.47; S, 11.42; Found: C, 53.36; H, 5.62; N, 12.36; S, 11.55.

Synthesis of 3,5-bis{5-[(oxiran-2-yl)methylthio]-1,3,4-oxadiazol-2-yl}-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (15). As pale white powders, mp 189–191°C; 2.68 g (54%). IR (KBr) ν: 3131 (NH), 1609, 1601 (4C=N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H, $2CH_3$), 2.62 (d, 4H, 2 oxiranyl-H, J = 4.2 Hz), 2.80 (m, 2H, 2 oxiranyl-H), 3.21 (d, 4H, 2 SCH_2, J = 4.6 Hz), 5.01 (s, 1H, pyridine-H), 7.10–7.26 (m, 5H, Ar–H), 9.20 (s, 1H, NH pyridine, exchangeable with D_2O). MS: *m/z* (%) = 497 (22) [M^+]. *Anal.* Calcd for $C_{23}H_{23}N_5O_4S_2$ (497.59): C, 55.52; H, 4.66; N, 14.07; S, 12.89; Found: C, 55.69; H, 4.59; N, 14.12; S, 12.95.

Synthesis of 3,5-bis(2-hydroxy)ethylthio)-1,3,4-oxadiazol-2-yl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (16). As pale brown crystals, mp 132–133°C; 1.70 g (36%). IR (KBr) ν: 3320 (2OH), 3125 (NH), 1612, 1605 (4C=N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.21 (d, 2H, 2OH, exchangeable with D_2O), 2.38 (s, 6H, $2CH_3$), 3.2 (t, 4H, $2CH_2$, J = 4.6 Hz), 3.9 (t, 4H, $2CH_2$, J = 4.4 Hz), 4.92 (s, 1H, pyridine-H), 7.13–7.29 (m, 5H, Ar–H), 9.26 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) (δ, ppm): 17.5, 17.9 ($2CH_3$), 43.6 (CH pyridine), 45.2, 62.6 (4 CH_2), 102.3, 103.2, 109.32, 112.28, 114.65, 121.6, 122.67, 131.82, 133.74, 140.3 (10 C-Ar), 153.5, 154.1, 155.3, 155.9 (4C=N). MS: *m/z* (%) = 473 (48) [M^+]. *Anal.* Calcd for $C_{21}H_{23}N_5O_4S_2$ (473.57): C, 53.26; H, 4.90; N, 14.79; S, 13.54; Found: C, 53.19; H, 5.01; N, 14.69; S, 13.62.

Biological activity. ***Antitumor screening.*** The aim of the present study was to illustrate the effect of some newly synthesized compounds on the human breast cancer cell line (T47D) in comparison with DOX in a trial to get more effective and less toxic agents.

MATERIALS AND METHODS

Preliminary experiments used the human tumor cell line to identify the potential toxicity of some newly synthesized compounds (**2a**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and **16**) in comparison with known anticancer drug DOX by using the method of Skehan *et al*. [28].

- Human tumor cell lines were obtained frozen in liquid nitrogen (−180°C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub culturing. RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA).
- The medium was prepared and used for culturing and maintenance of the human tumor cell lines. The prepared medium was kept in a refrigerator (4°C) and checked at regular intervals for contamination.
- Before the use, the medium was warmed at 37°C in a water bath and supplemented with penicillin/streptomycin and FBS.
- Different concentrations of the compounds tested (0, 5, 12.5, 25, and 50 μg/mL) were added to the cell monolayer. Each concentration was evaluated three times (each dose was incubated with the cells in three different wells).

- Monolayer cells were incubated with the compounds for 48 h at 37°C and atmosphere of 5% CO_2.
- After 48 h, cells were fixed, washed, and stained with Sulforhodamine-B stain.
- Excess stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an enzyme-linked immunosorbent assay reader.
- The relation between survival function and drug concentration is plotted to get the survival curve of tumor cell line after the specified compound.

Antimicrobial activity. The *in vitro* antimicrobial activity of the synthesized compounds was investigated against several pathogenic representative Gram-positive bacteria (*B. subtilis*) and (*S. aureus*), Gram-negative bacteria (*E. coli*), Fungi (*A. niger*) and Yeast (*C. albicans*).

Agar diffusion medium. Eleven compounds were screened *in vitro* for their antimicrobial activity by the agar diffusion method [29]. A suspension of organisms was added to a sterile nutrient agar medium at 45°C and the mixture was transferred to a sterile Petri dish and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer and filled with the solution of synthesized compounds (100 mg mL^{-1}). A hole filled with DMSO was used as control. The plates were left for 1 h at room temperature as a period of pre-incubation. The plates were then incubated at 37°C for 24 h and observed for antibacterial activity. Diameters of the zone of inhibition were measured and compared with that of the standard. Ciprofloxacin (50 mg mL^{-1}) and ketoconazole (50 mg mL^{-1}) were used as standards for antibacterial and antifungal activity, respectively. The observed zones of inhibition are presented in (Table 2).

Minimum inhibitory concentration. MIC of the test compounds was determined by the agar streak dilution method. Stock solutions of synthesized compounds were made using DMSO as a solvent. From this stock solution, a series of concentrations was prepared (0.075, 0.25, 0.45, and 0.65 mg mL^{-1}) and mixed with known quantities of molten sterile agar medium aseptically.

About 20 mL of the medium containing the tested compound was dispensed into a sterile Petri dish. Then, the medium was allowed to solidify. Micro-organisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 37°C for 24–48 h for antibacterial and antifungal activity, respectively. The lowest concentration of the synthesized compound that inhibits the growth of the given bacterium/fungus was considered as the MIC of the test compounds. The *MIC* values are tabulated in Table 3.

REFERENCES AND NOTES

[1] Sayed, H. H.; Morsy, E. M. H.; Flefel, E. M. Synth Comm 2010, 40, 1360.
[2] Sayed, H. H.; Flefel, E. M.; Abd El-Fattah, A. M.; El-Sofany, W. I.; Hassan, A. M. Egypt J Chem 2010, 55, 17.
[3] Sayed, H. H.; Morsy, E. M. H.; Kotb, E. R. Synth Comm 2010, 40, 2712.
[4] Rashad, A. E.; Sayed, H. H.; Shamroukh, A. H. Sulfur and Silicon 2005, 180, 2767.
[5] Amr, A. E.; Sayed, H. H.; Abdella, M. M. Arch Pharm Chem Life Sci 2005, 338, 433.
[6] Hafez, H. N.; Abbas, H. A. S.; El-Gazzar, A. R. B. A. Acta Pharm 2008, 58, 359.
[7] Abbas, H. A. S.; El Sayed, W. A.; Fathy, N. M. Eur J Med Chem 2010, 45, 973.
[8] Coburn, R. A.; Wierzba, M.; Suto, M. J.; Solo, A. J.; Triggle, A. M.; Triggle, D. J. J Med Chem 1988, 31, 2103.
[9] Komoda, H.; Inoue, T.; Node, K. Clin Exp Hypertens 2010, 32, 121.
[10] Aboraia, A. S.; Abdel-Rahman, H. M.; Mahfouz, N. H.; El-Gendy, M. A. Bioorg Med Chem 2006, 14, 1236.
[11] Akhtar, T.; Hameed, S.; Al-Masoudi, N. A.; Loddo, R.; Colla, P. L. Acta Pharm 2008, 58, 135.
[12] Kadi, A. A.; El-Brollosy, N. R.; Al-Deeb, O. A.; Habib, E. E.; Ibrahim, T. M.; El-Emam, A. A. Eur J Med Chem 2007, 42, 235.
[13] Zarghi, A.; Tabatabai, S. A.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A. Bioorg Med Chem Lett 2005, 15, 1863.
[14] El-Hamouly, W. S.; Kamelia, M. A.; Eman, M. H. A.; Eman, A. A. M. Egypt Pharm J (NRC) 2008, 7, 127.
[15] Nassar, I. F.; El-Assaly, S. A. Der Pharm Chem 2011, 3, 229.
[16] Tan, T. M. C.; Chen, Y.; Kong, K. H.; Bai, J.; Li, Y.; Lim, S. G.; Ang, T. H.; Lam, Y. Antivir Res 2006, 71, 7.
[17] Rollas, S.; Kucukguzel, S. G. Molecules 2007, 12, 1910.
[18] Rollas, S.; Gulerman, N.; Edeniz, H. II Farmaco 2002, 57, 171.
[19] Gokce, M.; Utku, S.; Kupeli, E. Eur J Med Chem 2009, 44, 3760.
[20] Silva, G. A.; Costa, L. M. M.; Brito, F. C. F.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. Bioorg Med Chem 2004, 12, 3149.
[21] Bijev, A. Lett Drug Des Discov 2006, 03, 506.
[22] Todeschini, A. R.; Miranda, A. L. P.; Silva, K. C.; Parrini, S. C.; Barreiro, E. J. Eur J Med Chem 1998, 33, 189.
[23] Debache, A.; Boulcina, R.; Belfaitah, A.; Rhouati, S.; Carboni, B. Synlett 2008, 4, 509.
[24] Turk, C.; Svete, J.; Golobic, A.; Golic, L.; Stanovnik, B. J Heterocycl Chem 1998, 35, 513.
[25] Svete, J.; Golic, L.; Stanovnik, B. J Heterocycl Chem 1997, 34, 1115.
[26] Shaban, M. A. E.; Taha, M. A. M.; Nasr, A. Z.; Morgaan, A. E. A. Pharmazie 1995, 50, 784.
[27] (a) Schaeffer, H. J.; Beauchamp, L. M.; De Miranda, P.; Ellon, G. B.; Bauer, D. J.; Collins, P. Nature 1978, 272, 583; (b) Liu, L. J.; Hong, J. H. Nucleosides Nucleotides 2009, 28, 303.
[28] Skehan, P.; Storeng, R. J Nat Canc Inst 1990, 82, 1107.
[29] Jain, S. R.; Kar, A. Planta Med 1971, 20, 118.