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Original article

Synthesis, *in vitro* antimicrobial and *in vivo* antitumor evaluation of novel pyrimidoquinolines and its nucleoside derivatives

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ABSTRACT

Seven series of pyrimidoquinoline derivatives have been synthesized, tetrazolo[4′,3′:-1,2]pyrimido[4,5-*b*]quinoline (**3**), 2-aminopyrimido[4,5-*b*]quinoline (**4**), triazolo[4′,3′:1,2]-pyrimidoquinoline (**5a,b**, **10**), imidazolo[3′,2′:1,2]pyrimido[4,5-*b*]-quinoline (**8a,b**), 6-chloro-2-methylthiopyrimido[4,5-*b*]quinoline (**12**), acetylated nucleosides (**16**, **17a**,**b**) and deacetylated nucleosides (**18**, **19a**,**b**). Some of the novel pyrimidoquinoline derivatives possess highly activity toward the bacteria and fungi species. The new quinolines derivatives were evaluated for their anticancer activity toward human cancer cell lines by the National Cancer Institute (NCI). Most of them had excellent growth inhibition activity, having LD_{50} values in the low micromolar to nanomolar concentration range.

1. Introduction

Quinolines and their derivatives are under constant investigation of the medicinal chemists in view of their profound range of biological activities, antimalarial [1], antibacterial [2], anti-asthmatic [3], antihypertensive [4] anti-inflammatory [5]. The quinoline structural moiety is present in most of the antibacterials introduced in the recent past namely, nalidixic acid [6], norfloxacin [7], ciprofloxacene [8], ofloxacin [9]. Also imiquimed, clioquinol, and enoxacin are antiviral, antifungal and antibacterial drugs which contain pyrimidine and quinolines rings. Moreover, pyrimidoquinoline is present in a number of biologically active organic compounds [10–14] which exhibit, antibacterial [15], anticancer [16], medicinal [17,18], and anti-inflammatory activity.

N-Glycosides have received considerable attention, because they are widely employed as biological inhibitors [19–23], inducers [24] and ligands [25–27] for affinity chromatography of carbohydrate-processing enzymes and proteins. Moreover, they are promising candidates in synthetic carbohydrate chemistry as convenient and versatile glycosyl donors [28]. Among these glycosyl donors are the *N*-glycosyl heterocycles that are sufficiently stable under a variety of reaction conditions and have the ability to be readily converted into a variety of other functionalities. On the other hand, it was reported that dihydropyridine glycosides were used as P-glycoprotein (Pgp) substrates or inhibitors in the protein glycosylation process [29]. Moreover, the increasing biological importance of pyrimidoquinoline derivatives, particularly in the field of chemotherapy, prompted us to develop and identify the new molecules, such as tetrazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinoline, triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinoline, imidazolo[3′,2′:1,2]pyrimido[4,5-*b*]-quinoline, and its *N*-glycosides derived from pyrimido[4,5-*b*]quinoline-4,6-dione in order to investigate the effect of such structural modification on the antimicrobial and antitumor activities.

2. Results and discussion

2.1. Chemistry

As part of our programme of research on the synthesis of pyridoquinolines and biological activity of these types of compounds, we became interested in the synthesis of the corresponding glycosides analogues [30–33] in order to study their antimicrobial and antitumor activities. Thus, 5-aryl-8,8-dimethyl-2-methylthio-5,8,9,10-tetrahydropyrimido[4,5-*b*]-quinoline-4,6-dione (**1a**–**c**) were prepared in good yield via the one–pot reaction of 6-amino-2-methylthiopyrimidin-4-one with 5,5-dimethyl-cyclohexane-1,3-dione and aryl-carboxal-dehydes [34]. Compounds (**1a**–**c**) treated

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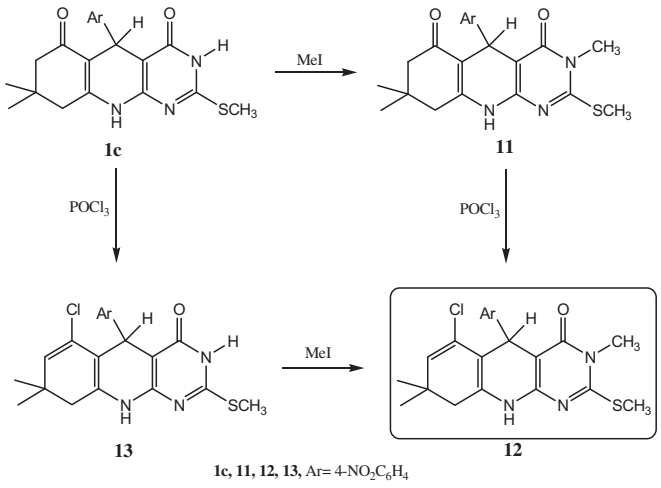
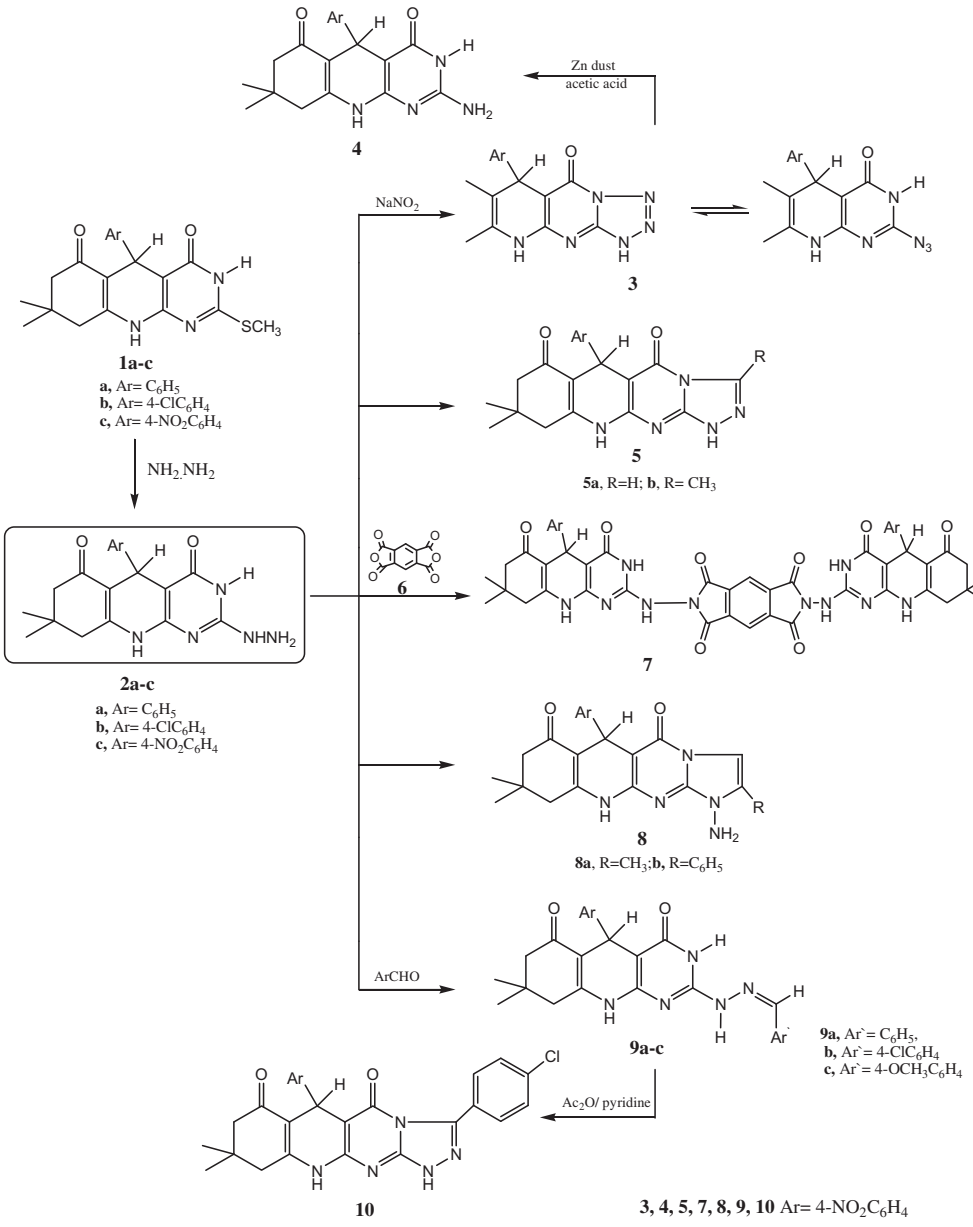
with hydrazine hydrate (99%) (Scheme 1), afforded 5-aryl-8,8-dimethyl-2-hydrazino-5,8,9,10-tetrahdropyeimido[4,5-*b*]quinoline-4,6-dione (**2a**–**c**). Compound **2c** could be used as a starting material for the synthesis of new polynuclear heterocycles such as tetrazolopyrimido[4,5-*b*]quinoline. Thus treatment of **2c** with nitrous acid led to the formation of 5,8,9,10-tetrahydrotetrazolo-[4′,3′:1,2]pyrimido[4,5-*b*]quinoline-4,6-dione derivative (**3**) (Scheme 1), which is the tautomer of 2-azido-pyrimido[4,5-*b*]quinoline. The IR spectrum of **3** displayed absorption bands at 3254 (NH's) and 1715, 1683 cm^{-1} (2C=O) and characteristic absorption band for the azido group [35] at 2318 cm^{-1}.

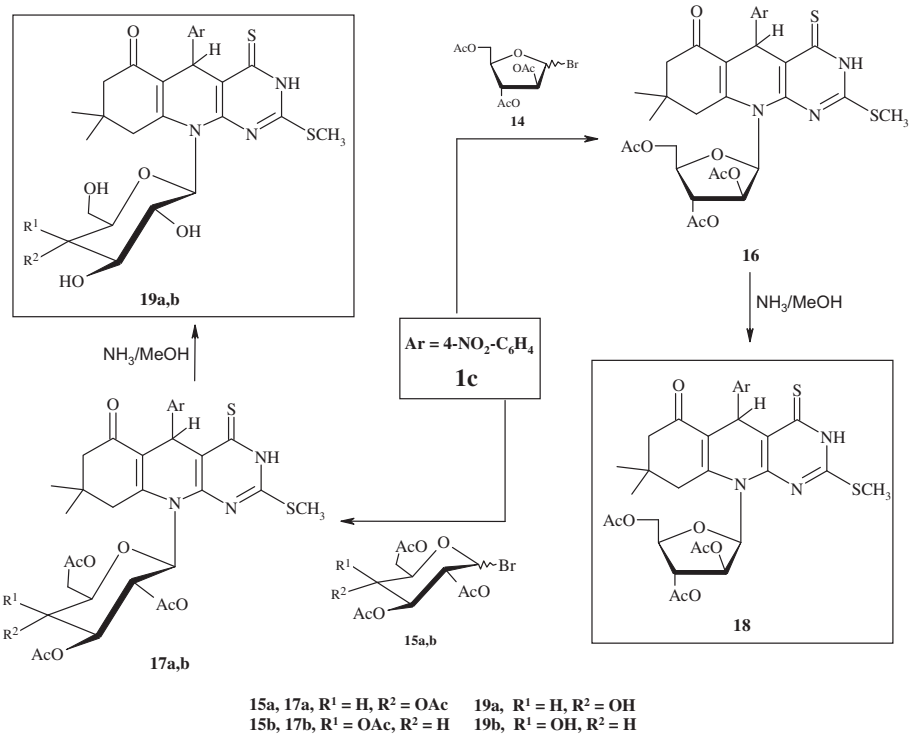
According to the literature aminopyrimidines have biological activities as anticancer, antibacterial, and antimalarial [36]. Therefore, compound **3** was reduced into 2-amino-pyrimido[4,5-*b*]quinoline **4** by zinc dust. Heating the hydrazine derivative **2c** with aliphatic acids, namely formic or acetic acid, resulted in the formation of triazolo[4′,3′:1,2]pyrimido-[4,5-*b*]quinoline **5a**,**b**



Scheme 2.



Scheme 1.



Scheme 3.

(Scheme 1). ^{1}H NMR spectrum of **5b**, as an example, shows three signals at δ 0.90, 1.01, 1.12 for ($3CH_3$) and disappearance of signals at δ 12.0 (3NH).

Cyclocondensation of compound **2c** with acid anhydride (**6**) afforded the corresponding 2,6-bis{8,8-dimethyl-5-(4-nitrophenyl)-3,4,5,6,7,8,9,10-octahydrpyrimido[4,5-*b*]quinoline-2-yl-amino-4,6-dione}pyrrolo[3,4-*f*]isoindole-1,3,5,7-tetraone (**7**). In addition, the reaction of **2c** with α-haloketones namely, chloroacetone or phenacyl bromide in dry xylene, yielded the respective imidazolo [3′,2′:1,2]pyrimido[4,5-*b*]quinoline **8a**,**b** as shown in (Scheme 1). IR spectrum of **8a** displayed absorption bands around 3318, 1715, 1687 cm^{-1} corresponding to (NH's) and (2C=O) groups, respectively.

Table 1
Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds.

Compds.	Microorganism inhibition zone diameter (mm)					
	Gram+ve bacteria		Gram−ve bacteria		Fungi	
	Bacillus subtilis	*Streptococcus lactis*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Candida gabrata*
1c	13	14	13	14	7	8
2c	15	15	15	15	9	8
3	23	18	20	19	12	10
4	25	20	27	23	9	9
5a	20	20	19	18	9	9
5b	18	21	18	22	11	11
7	22	25	23	22	13	12
8a	19	22	24	23	9	10
8b	20	20	23	23	8	6
9a	17	18	17	16	7	6
9b	16	17	18	17	7	6
9c	15	16	17	16	7	8
10	18	15	18	17	9	7
11	15	13	15	14	8	6
12	14	13	15	14	10	9
13	14	14	15	13	8	6
16	19	20	21	23	8	9
17a	20	20	23	22	8	8
17b	20	19	25	23	7	8
18	20	18	24	23	7	8
19a	21	19	23	23	8	9
19b	21	20	22	22	8	7
Nystatin	–	–	–	–	20	20
Nalidixic acid	25	25	25	25	–	–

Inhibition zone, 6–10 mm slight activity, 11–15 mm moderate activity, more than 15 mm high activity.

Table 2
MIC in mg/ml of the newly synthesized compounds.

Compds.	Gram+ve bacteria		Gram−ve bacteria		Fungi	
	Bacillus subtilis	*Streptococcus lactis*	*Escherichia coli*	*Pseudomonas aeuroginosa*	*Candida albicans*	*Candida gabrata*
3	30	30	30	30	40	40
4	20	20	20	20	40	40
5a	40	40	40	40	40	40
5b	30	30	30	30	40	40
7	30	30	30	30	40	40
8a	30	30	30	30	50	50
8b	30	30	30	30	50	50
9a	20	20	20	20	40	40
9b	20	20	20	20	40	40
9c	20	20	20	20	40	40
10	30	30	30	30	30	30
16	30	30	30	30	30	30
17a	30	30	30	30	30	30
17b	30	30	30	30	30	30
18	30	30	30	30	30	30
19a	30	30	30	30	30	30
19b	30	30	30	30	30	30

Also, ^{1}H NMR spectrum of **8a**, showed signals at δ 0.89 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.95 (m, 4H, $2CH_2$), 2.08 (s, 3H, CH_3), 5.03 (s, 1H, C5-*H*), 7.46 (d, $J = 8.1$ *Hz*, 2H, Ar-*H*), 7.97 (d, $J = 8.1$ *Hz*, 2H, Ar-*H*), 8.16 (s, 1H, imidazole proton), 9.12, 9.84 (2br, 3H, NH, NH_2, D_2O exchangeable).

According to El-Gazzar et al. [37] 2-hydrazino derivative **2c** gave the 2-(aryl-methylenehydrazone)pyrimido[4,5-*b*]quinoline derivatives **9a**–**c** (Scheme 1) when treated with the appropriate aldehyde. Compounds **9a**–**c** gave compatible spectral and analytical data (cf. Exp.). The arylhydrazone **9b** could be cyclized into the corresponding 1,2,4-triazolopyrimido[4,5-*b*]quinoline **10**, when reflexed in a mixture of acetic anhydride/pyridine (2:1). The alkylation of **1c** was carried out by treatment with one equivalent of methyl iodide in the presence of sodium ethoxide to give 3,8,8-trimethyl-2-methylthio-5-(4-nitrophenyl)-5,8,9,10-tetrahydropyrimido[4,5-*b*]quinoline-4,6-dione (**11**) (Scheme 2). The structure was confirmed by spectroscopic data. ^{1}H NMR spectrum of **11**, showed signals at δ 0.93 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.04 (d, $J = 17.1$ *Hz*, 2H, CH_2), 2.15 (d, $J = 17.1$ *Hz*, 2H, CH_2), 2.56 (s, 3H, SCH_3), 3.26 (s, 3H, NCH_3), 5.00 (s, 1H, C5-*H*), 7.45 (d, $J = 7.9$ *Hz*, 2H, Ar-*H*), 8.05 (d, $J = 8.0$ *Hz*, 2H, Ar-*H*), 9.92 (br, 1H, NH, D_2O exchangeable). Compound **11** was converted into the corresponding 6-chloro-pyrimido[4,5-*b*]quinoline-4,6-dione **12** by refluxing in phosphorus oxychloride. Beside the correct values in elemental analyses and spectral data (cf. Exp.), the structure of compound **12** was established chemically upon treatment of **1c** with $POCl_3$ to give the corresponding 6-chloro derivative **13** which was alkylated with methyl iodide in the presence of sodium ethoxide to give **12** as shown in (Scheme 2).

However the glycosylation of **1c** with 2,3,5-tri-*O*-acetyl-β-D-arabinopyranosyl bromide **14** or 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl bromide **15a**,**b** in acetone and in the presence of aqueous potassium hydroxide afforded the corresponding acetylated nucleosides **16**, **17a**,**b** respectively in good yields (57–64%) (Scheme 3). Thin layer chromatography (chloroform: methanol, 8:2) indicated the formation of the pure compounds. The structures of **16**, **17a**,**b** were confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, ^{13}C NMR) (cf. Exp.). The ^{1}H NMR spectrum of compound **17a** as an example, showed the anomeric proton of the glucose moiety as a doublet at δ 6.08 ppm with a coupling constant $J_{1'-2'} = 10.9$ Hz indicating β-configuration of the anomeric center. The other protons of the glucopyranose ring resonated at δ 3.71–5.34 ppm, while the four acetoxy groups appeared as four singlets at δ 2.02–2.15 ppm. The ^{13}C NMR revealed the signals at δ 169.35–170.45, 194.36 ppm due to the presence of (6CO), signals at δ 61.98, 68.67, 70.32, 71.03, 71.18, 71.52 ppm are assigned to C-6′,

Table 3
Effect of treatment with different compounds on the survival of ascites tumor harboring mice inoculated with (HEPG2) or (HELA) cells line.

Conc. of Compds. (μg/kg b. w.)	Survival time (days) HEPG2	% increase in life span of animals HEPG2	Survival time (days) HELA	% increase in life span of animals HELA
Control	22.00 ± 1.90	0	22.00 ± 1.90	0
Cisplatine (2 mg/kg b. w.)	45.00 ± 4.20[a]	84	45.00 ± 4.20[a]	83
2c (10 μg/kg b. w.)	28.00 ± 2.30[a]	31	29.00 ± 2.50[a]	38
3 (10 μg/kg b. w.)	29.00 ± 2.50[a]	29	27.00 ± 1.90[a]	35
4 (10 μg/kg b. w.)	35.00 ± 1.90[a]	49	31.00 ± 2.70[a]	50
5a (10 μg/kg b. w.)	31.00 ± 2.70[a]	55	30.00 ± 2.20[a]	51
7 (10 μg/kg b. w.)	38.00 ± 2.50[a]	58	29.00 ± 2.20[a]	53
8a (10 μg/kg b. w.)	37.00 ± 2.20[a]	56	34.00 ± 2.50[a]	51
9b (10 μg/kg b. w.)	37.00 ± 2.70[a]	53	32.00 ± 2.70[a]	50
10 (10 μg/kg b. w.)	36.00 ± 2.50[a]	51	34.00 ± 2.50[a]	57
11 (10 μg/kg b. w.)	34.00 ± 2.50[a]	50	28.00 ± 2.30[a]	48
12(10 μg/kg b. w.)	33.00 ± 2.30[a]	48	31.00 ± 1.90[a]	45
13 (10 μg/kg b. w.)	33.00 ± 1.90[a]	47	30.00 ± 2.70[a]	47
16 (10 μg/kg b. w.)	35.00 ± 2.70[a]	62	36.00 ± 1.90[a]	66
17a (10 μg/kg b. w.)	36.00 ± 1.90[a]	66	37.00 ± 2.80[a]	61
18 (10 μg/kg b. w.)	37.00 ± 2.30[a]	60	38.00 ± 2.50[a]	66
19a (10 μg/kg b. w.)	38.00 ± 2.50[a]	60	36.00 ± 2.70[a]	61

Values are mean ± SE.; $n = 10$ mice; [a]$P < 0.05$ significant with respect to control.

Table 4
In vivo acute toxicity of prepared compounds LD_{50} μg/kg b.w.

Compounds	LD_{50} μg/kg b.w. (HEPG2)	LD_{50} μg/kg b.w. (HELA)
2c	89	100
3	90	90
4	95	90
5a	100	98
7	99	99
8a	93	90
9b	96	90
10	95	89
11	98	111
12	100	103
13	101	110
16	104	114
17a	109	111
18	110	116
19a	115	119

C-4′, C-2′, C-3′, C-5′ and C-1′ respectively. Also, the four signals at δ 20.81–20.99 ppm are assigned to the acetate methyl carbon atoms.

Deacetylation of acetylated nucleosides **16**, **17a**,**b** using saturated solution of ammonia in methanol at room temperature afforded the corresponding deacetylated nucleosides **18**, **19a**,**b** respectively. The structures of free nucleosides **18** and **19a**,**b** have been established on the basis of their spectral data and elemental analyses. Thus, the ^{1}H NMR spectrum of **19a** showed the anomeric proton as a doublet at δ 6.10 ppm. $J_{1'-2'} = 10.8$ *Hz* indicating a β-D-configuration. The signals of the other six glucose protons appeared at δ 3.25–4.21 ppm, while the signals that disappear on rapid exchange with D_2O are observed at δ 4.60–5.13 ppm, were assigned as the four hydroxyl groups.

2.2. Pharmacological screening

2.2.1. Antimicrobial activity

In vitro antimicrobial activity: *In vitro* antimicrobial screening of the new synthesized bases and glycoside compound was evaluated against two Gram-positive bacteria (*Bacillus subtilis* and *Streptococcus lactis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) and two fungal strains (*Candida albicans* and *Candida gabrata*). Activities of the tested compound (Table 1) were evaluated by agar diffusion method. Compound **4** exhibited excellent activity toward Gram-positive bacteria, while exhibited highly activity toward Gram-negative bacteria as compared with the reference drug (Nalidixic Acid) due to the presence of amino group. Compounds **3**, **7**, **8a**, **8b** and **16**–**19** exhibited good activity towards Gram-negative and Gram-positive bacteria, but compounds **1c**, **2c**, **5a**, **5b** and **9**–**13** exhibited moderate activity towards Gram-negative and Gram-positive bacteria, while most of the newly synthesized compounds exhibited moderate activity against fungi as compared with the reference drug (Nystatin).

The MIC (minimum inhibitory concentration) of the most active compounds toward the microorganism showed that the MICs ranged between 20 and 30 μg/disk. The activity is tested at concentration of 50 μg/disk. The minimum inhibitory concentrations (MIC) for compounds with high activity are presented in (Table 2) were in accordance with the results obtained in the primary screening.

2.2.2. Antitumor activity

The synthesized compounds were tested and evaluated for inhibition using human liver carcinoma cell line (HEPG2) and Human cervix carcinoma cell line (HELA) Table 3. The administration of prepared compounds significantly increases ($P < 0.05$) the life span of the animals as compared with control group [animals injected with Human liver carcinoma cell line (HEPG2) or Human cervix carcinoma cell line (HELA) alone]. There is a gradual increase in life span using the prepared compounds. The increase in life span was around 29%–66%. The standard reference drug (Cisplatine 2 mg/kg body weight) exhibited 84% ($P < 0.05$) increase in life span of the animals. All the animals in the HEPG2 or HELA injected alone group were dead after 25 or 30 days respectively.

The results of measurements of effect of treatment with tested compounds on the survival of ascites tumor harboring mice inoculated with (HEPG2) cells line (Table 3) revealed that compound **17a** was the most active exhibiting 66% increase in life span followed by compounds **16**, **18** and **19a** (62–60%). Compounds **7**, **8a**, **9b**, **10** and **11** (50–58%) showed moderate activity against the same tumor cell lines. While the results with (HELA) cells line (Table 3) showed that compounds **16** and **18** showed the highest activity with an increase in life span 66% followed by compounds **17a** and **19a** (61%). Compounds **10**, **7**, **5a**, **8a**, **4** and **9b** showed moderate activity with increase in life span 50–57%.

On the other hand, the results of *In vivo* acute toxicity measurements of synthesized compound with respect to ascites tumor harboring mice inoculated with (HEPG2) cells (Table 4) revealed that compound **19a** exhibiting the highest LD_{50} with 115 μg/kg b.w. followed by compounds **18**, **17a**, **16**, **13**, **5a** and **12** (LD_{50} 100–110 μg/kg b.w.) among the tested series of compounds. For the ascites tumor harboring mice inoculated with (HELA) cells, compound **19a** also showed the highest LD_{50} with 119 μg/kg b.w.

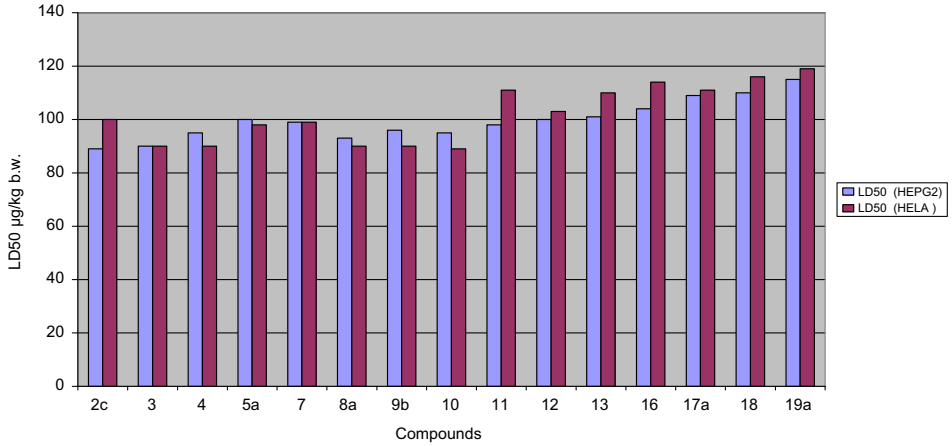


Fig. 1. Acute toxicity of synthesized compounds (LD_{50} μg/kg b.w.).

followed by compounds **18**, **16**, **17a**, **11** and **13** with acute toxicities (LD_{50} 110–116 μg/kg b.w.) Fig. 1.

3. Conclusion

The overall results indicated that, the tested compounds showed promising antimicrobial activity against bacteria and Fungi. Among the tested compounds, for gram-positive and gram-negative bacteria, it was noticed compound **4** demonstrated inhibitory activities more than compounds **3**, **7**, **8a**, **8b** and **16**–**19**, while most of the newly synthesized compounds exhibited moderate activity against fungi as compared with the reference drug (Nystatin). On the other hand, the antitumor activities revealed that the acetylated sugar (**16**, **17a**) and deacetylated sugar (**18**, **19a**) most significant activities than the other tested compounds.

4. Experimental

4.1. Chemistry

All melting points were measured on ElectrothermalIA 9100 series digital melting point apparatus. Microanalytical data (in accord with the calculated values) were performed by Vario, Elementar apparatus (Shimadzu). The IR spectra (KBr) were recorded on a Perkin–Elmer 1650 spectrometer (USA). ^1H and ^{13}C NMR spectra were determined on a JEOL EX-300 and JEOL ECA-500. Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standards and DMSO-d_6 as solvent. Mass spectra were recorded on 70 ev EI Ms-QP 1000 EX (Shimadzu, Japan). Antimicrobial activities were measured at pharmacology department, National Research Center, Cairo, Egypt. Antitumor activities were measured at the National Cancer Institute, Cairo University, Cairo, Egypt.

4.1.1. 5-Aryl-8,8-dimethyl-2-hydrazino-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**2a**–**c**)

General procedure. A suspension of compound **1a**–**c** (0.01 mol) in hydrazine hydrate (99%, 20 ml) was stirred under reflux for 10 h. The reaction mixture was allowed to cool to room temperature. The solid precipitate was filtered off, washed with ethanol, dried and crystallized from dimethylformamide to produce **2a**–**c** in high yields.

*4.1.1.1. 2-Hydrazino-8,8-dimethyl-5-phenyl-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**2a**).* It was obtained from **1a** (3.7 g, 0.01 mol), as a yellow powder, crystallized from DMF in a 75% yield, mp 319–320 °C; IR (cm^{-1}, υ): 3280 (br, NH's), 1703, 1682 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.82 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 2.12 (d, *J* = 17.0 *Hz*, 2H, CH_2), 2.33 (d, *J* = 17.0 *Hz*, 2H, CH_2), 4.96 (s, 1H, C5-*H*), 7.42 (m, 2H, Ar-*H*), 7.76 (m, 3H, Ar-*H*), 8.55, 9.95, 12.30 (3br, 5H, 3NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 351 (M^+, 78%); $C_{19}H_{21}N_5O_2$ (351.40); Requires (Found): C, 64.94 (65.01); H, 6.02 (5.98); N, 19.93 (19.85).

*4.1.1.2. 5-(4-Chlorophenyl)-2-hydrazino-8,8-dimethyl-5,8,9,10-tetrahydropyrimido[4,5-b] quinoline-4,6-dione (**2b**).* It was obtained from **1b** (4.0 g, 0.01 mol), as a yellow powder, crystallized from DMF in a 78% yield, mp 310–311 °C; IR (cm^{-1}, υ): 3290 (br, NH's), 1712, 1675 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.95 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.96 (d, *J* = 16.9 *Hz*, 2H, CH_2), 2.14 (d, *J* = 17.0 *Hz*, 2H, CH_2), 5.05 (s, 1H, C5-*H*), 7.44 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 8.03 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 8.15, 9.90, 12.27 (3br, 5H, 3NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 385 (M^+, 90%), 386 ($M^+ + 1$, 18%), 387 ($M^+ + 2$, 31%); $C_{19}H_{20}ClN_5O_2$ (385.85); Requires (Found): C, 59.14 (59.10); H, 5.22 (5.30); N, 18.15 (18.20).

*4.1.1.3. 2-Hydrazino-8,8-dimethyl-5-(4-nitrophenyl)-5,8,9,10-terahydropyrimido[4,5-b] quinoline-4,6-dione (**2c**).* t was obtained from 1c (4.12 g, 0.01 mol), as a yellow powder, crystallized from DMF in a 90% yield, mp 322–323 °C; IR (cm^{-1}, υ): 3288 (br, NH's), 1710, 1687 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.88 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 2.01 (d, *J* = 17.2 *Hz*, 2H, CH_2), 2.22 (d, *J* = 17.1 *Hz*, 2H, CH_2), 4.93 (s, 1H, C5-*H*), 7.47 (d, *J* = 8.3 *Hz*, 2H, Ar-*H*), 8.09 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.17, 9.92, 12.0 (3br, 5H, 3NH, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 27.68, 29.90 ($2CH_3$), 35.48, 39.82 (C-5 + C-8), 41.07, 51.0 ($2CH_2$), 91.58, 109.62, 123.80, 123.91, 129.60, 129.63, 146.35, 153.06, 154.99 (Ar-10C), 156.16 (C=N), 157.12, 194.74 (2C=O); Its MS (*m*/*z*), 396 (M^+, 51%); $C_{19}H_{20}N_6O_4$ (396.40); Requires (Found): C, 57.57 (57.50); H, 5.09 (4.99); N, 21.20 (21.28).

4.1.2. 8,8-Dimethyl-5-(4-nitrophenyl)-5,8,9,10-tetrahydrotetrazolo[4′,3′:1,2]pyrimido[4,5-b] quinoline-4,6-dione (**3**)

To an ice-cold solution of compound **2c** (3.96 g, 0.01 mol) in acetic acid (10 ml) was added drop wise a solution of sodium nitrite (10.35 g, 0.15 mol) in a least amount of water in an ice bath at −5 °C. The reaction mixture was allowed to stand overnight at room temperature, then poured onto water (100 ml). The solid precipitated was filtered off and crystallized from ethanol to produce **3** as orange powder in a 60% yield, mp 335–337 °C; IR (cm^{-1}, υ): 3254 (br, NH's), 1715, 1683 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.02 (d, *J* = 16.8 *Hz*, 2H, CH_2), 2.14 (d, *J* = 16.9 *Hz*, 2H, CH_2), 5.01 (s, 1H, C5-*H*), 7.47 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.08 (d, *J* = 8.3 *Hz*, 2H, Ar-*H*), 9.79, 10.14 (2br, 2H, 2NH, D_2O exchangeable); Its MS (*m*/*z*), 407 (M^+, 45%); $C_{19}H_{17}N_7O_4$ (407.38); Requires (Found): C, 56.01 (56.12); H, 4.21 (4.18); N, 24.07 (24.11).

4.1.3. 2-Amino-8,8-dimethyl-5-(4-nitrophenyl)-5,8,9,10-tetrahydropyrimido[4,5-b] quinoline-4,6-dione (**4**)

To a well-stirred solution of the appropriate tetrazolo-pyrimido [4,5-*b*]quinoline **3** (4.07 g, 0.01 mol) in glacial acetic acid (30 ml) was added portionwise activated zinc dust (5.0 g) at room temperature over a period of 30 min. Stirring was continued for additional 3 h. Then the reaction mixture was heated on water bath (80–90 °C) for 3 h. The progress of reduction was monitored by TLC. After allowing the reaction mixture to cool to room temperature, it was poured onto cold water (100 ml). The insoluble solid which separated was filtered, washed with water, and dried. The crude solid was extracted with hot diethyl ether and the solid obtained after the removal of ether under reduced pressure was crystallized from ethanol, as brown powder in a 41% yield, mp 301–302 °C; IR (cm^{-1}, υ): 3325 (br, NH's), 1700, 1680 (2C=O); ^1HNMR (DMSO-d_6) (δ, ppm) δ 0.92 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 2.10 (m, 4H, $2CH_2$), 5.04 (s, 1H, C5-*H*), 7.56 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.02 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.50, 9.90, 11.03 (3br, 4H, 2NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 381 (M^+, 12%); $C_{19}H_{19}N_5O_4$ (381.39); Requires (Found): C, 59.84 (59.79); H, 5.02 (4.98); N, 18.36 (18.33).

*4.1.3.1. 8,8-Dimethyl-5-(4-nitrophenyl)-5,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido [4,5-b]quinoline-4,6-dione (**5a**).* A mixture of **2c** (3.96 g, 0.01 mol), formic acid (10 ml), and 1 ml of concentrated hydrochloric acid was heated under reflux for 6 h. The reaction mixture was allowed to cool to room temperature and poured onto water (100 ml). The formed solid was collected by filtration, washed with ethanol (20 ml), dried, and crystallized from ethanol, as yellow powder in a 76% yield, mp 299–300 °C; IR (cm^{-1}, υ): 3290 (br, NH's), 1698, 1679 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.98 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.99 (d, *J* = 17.3 *Hz*, 2H, CH_2), 2.15 (d, *J* = 17.3 *Hz*, 2H, CH_2), 5.11 (s, 1H, C5-*H*), 7.43 (d, *J* = 7.9 *Hz*, 2H, Ar-*H*), 8.03 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 8.64 (s, 1H, azomethine proton), 9.90, 10.0 (2br, 2H, 2NH, D_2O exchangeable); Its MS (*m*/*z*), 406 (M^+,

43%); $C_{20}H_{18}N_6O_4$ (406.39); Requires (Found): C, 59.11 (59.05); H, 4.46 (4.40); N, 20.68 (20.73).

*4.1.3.2. 3,8,8-Trimethyll-5-(4-nitrophenyl)-5,8,9,10-tetrahydro[1,2,4] triazolo[4′,3′:1,2] pyrimido[4,5-b]quinoline-4,6-dione (**5b**).* A mixture of **2c** (3.96 g, 0.01 mol) and glacial acetic acid (30 ml) was stirred under reflux for 6 h (TLC). The reaction mixture was allowed to cool to room temperature and then poured onto water (100 ml). The solid formed was collected by filtration, washed with ethanol (20 ml), dried and crystallized from ethanol, as yellow powder in a 69% yield, mp 276–278 °C; IR (cm^{-1}, υ): 3291 (br, NH's), 1709, 1681 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.90 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.02 (d, J = 16.9 *Hz*, 2H, CH_2), 2.22 (d, J = 16.9 *Hz*, 2H, CH_2), 4.94 (s, 1H, C5-*H*), 7.44 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.08 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.84, 9.71 (2br, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 21.38, 27.74, 29.83 ($3CH_3$), 33.05, 39.78 (C-5 + C-8), 41.04, 50.99 ($2CH_2$), 93.15, 109.63, 123.98, 129.65, 129.84, 146.43, 153.03, 154.59, 155.79 (Ar-10C), 159.9, 162.56 (2C=N), 168.94, 194.82 (2C=O); Its MS (*m*/*z*), 420 (M^+, 46%), 421 (M^+ + 1, 14%); $C_{21}H_{20}N_6O_4$ (420.42); Requires (Found): C, 59.99 (60.03); H, 4.79 (4.72); N, 19.99 (20.02).

*4.1.4. 2,6-Bis{8,8-dimethyl-5-(4-nitrophenyl)-3,4,5,6,7,8,9,10-octahydropyrimido[4,5-b] quinoline-2-yl-amino-4,6-dione}pyrrolo [3,4-f]isoindole-1,3,5,7-tetraone (**7**)*

A mixture of compound **2c** (7.38 g, 0.02 mol) and 1,2,4,5-benzene-tetracarboxylic dianhydride **6** (2.18 g, 0.01 mol) in glacial acetic acid (30 ml) was heated under reflux for 6 h. The residue formed was filtered off and crystallized from ethanol, as yellow powder in a 68% yield, mp 323–325 °C; IR (cm^{-1}, υ): 3339 (br, NH's), 1711–1675 (8C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.89 (s, 6H, $2CH_3$), 1.01 (s, 6H, $2CH_3$), 1.96 (d, J = 17.3 *Hz*, 4H, $2CH_2$), 2.46 (d, J = 17.3 *Hz*, 4H, $2CH_2$), 4.94 (s, 2H, 2C5-*H*), 7.44 (d, J = 8.2 *Hz*, 4H, Ar-*H*), 8.01 (d, J = 8.2 *Hz*, 4H, Ar-*H*), 8.10 (d, J = 7.9 *Hz*, 2H, Ar-*H*), 8.80, 9.65, 11.3 (3br, 6H, 6NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 21.83, 21.99, 27.75, 29.83 ($4CH_3$), 33.06, 36.01, 39.77, 39.98 (2C-5 + 2C-8), 40.81, 41.02, 49.95,50.00 ($4CH_2$), 109.63, 123.99, 124.12, 129.66, 130.09, 130.56, 135.65, 146.44, 153.03, 154.32 (Ar-26C), 155.98, 156.21 (2C=N), 168.23, 168.35, 168.49, 169.92, 194.83 (8C=O); Its MS (*m*/*z*), 974 (M^+, 58%); $C_{48}H_{38}N_{12}O_{12}$ (974.89); Requires (Found): C, 59.14 (59.10); H, 3.93 (3.98); N, 17.24 (17.29).

*4.1.5. 1-Amino-8,8-dimethyl-2-(methyl or phenyl)-5-(4-nitrophenyl)-5,8,9,10-tetrahydro-imidazolo[3′,2′:1,2]pyrimido [4,5-b]quinoline-4,6-dione (**8a**,**b**)*

General procedure. A mixture of compound **2c** (3.96 g, 0.01 mol) and chloroacetone or phenacylbromide (0.01 mol) was heated under reflux for 5 h in dry xylene (30 ml). The solid that separated upon cooling was filtered off and crystallized from appropriate solvent to produce **8a** and **8b** respectively in high yields.

*4.1.5.1. 1-Amino-2,8,8-trimethyl-5-(4-nitrophenyl)-5,8,9,10-tetrahydroimidazolo[3′,2′:1,2] pyrimido[4,5-b]quinoline-4,6-dione (**8a**).* It was obtained from compound **2c** (3.96 g, 0.01 mol) and chloroacetone (0.92 g, 0.01 mol), crystallized from ethanol, as white crystals in a 62% yield, mp 298–299 °C; IR (cm^{-1}, υ): 3318 (br, NH's), 1715, 1687 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.89 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.95 (m, 4H, $2CH_2$), 2.08 (s, 3H, CH_3), 5.03 (s, 1H, C5-*H*), 7.46 (d, J = 8.1 *Hz*, 2H, Ar-*H*), 7.97 (d, J = 8.1 *Hz*, 2H, Ar-*H*), 8.16 (s, 1H, imidazole proton), 9.12, 9.84 (2br, 3H, NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 434 (M^+, 12%); $C_{22}H_{22}N_6O_4$ (434.45); Requires (Found): C, 60.82 (60.78); H, 5.10 (5.05); N, 19.34 (19.40).

*4.1.5.2. 1-Amino2-phenyl-5-(4-nitrophenyl)-8-dimethyl-5,6,7,8,9,10-hexahydro[1,3] imidazolo[3′,2′:1,2]pyrimido[4,5-b]quinoline-4,6-dione (**8b**).* It was obtained from compound **2c** (3.96 g, 0.01 mol) and phenacylbromide (1.97 g, 0.01 mol), crystallized from methanol, as yellow powder in a 66% yield, mp 308–310 °C; IR (cm^{-1}, υ): 3305 (br, NH's), 1712, 1679 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.95 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.96 (d, J = 16.9 *Hz*, 2H, CH_2), 2.15 (d, J = 16.8 *Hz*, 2H, CH_2), 4.95 (s, 1H, C5-*H*), 7.30 (m, 3H, Ar-*H*), 7.39 (d, J = 8.2 *Hz*, 2H, Ar-*H*), 8.05 (s, 1H, imidazole proton), 8.13 (d, J = 8.2 *Hz*, 2H, Ar-*H*), 8.22 (m, 2H, Ar-*H*), 9.10, 9.87 (2br, 3H, NH, NH_2, D_2O exchangeable); Its MS (*m*/*z*), 496 (M^+, 28%); $C_{27}H_{24}N_6O_4$ (496.52); Requires (Found): C, 65.31 (65.25); H, 4.87 (4.92); N, 16.93 (16.85).

*4.1.6. 2-(Arylmethylenhydrazone)-5-(4-nitrophenyl)-8,8-dimethyl-5,8,9,10-tetrahydro-pyrimido[4,5-b]quinoline-4,6-dione (**9a**–**c**)*

General procedure. A mixture of **2c** (3.96 g, 0.01 mol), the appropriate aromatic aldehyde (0.01 mol) was stirred under reflux in glacial acetic acid (30 ml) for 50 min. The reaction mixture was allowed to cool to room temperature; the formed solid was filtered off and crystallized from appropriate solvent to produce **9a**–**c**.

*4.1.6.1. 2-[N′-(Phenylmethylen)-hydrazone]-8,8-dimethyl-5-(4-nitrophenyl)-5,8,9,10-tetra-hydropyrimido[4,5-b]quinoline-4,6-dione (**9a**).* It was obtained from compound **2c** (3.96 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol), crystallized from acetic acid, as yellow powder in a 58% yield, mp 323–325 °C; IR (cm^{-1}, υ): 3298 (br, NH's), 1710, 1687 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.83 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.89 (d, J = 16.8 *Hz*, 2H, CH_2), 2.16 (d, J = 16.8 *Hz*, 2H, CH_2), 4.98 (s, 1H, C5-*H*), 7.42 (m, 3H, Ar-*H*), 7.57 (d, J = 8.2 *Hz*, 2H, Ar-*H*), 7.94 (d, J = 8.2 *Hz* 2H, Ar-*H*), 8.08 (m, 2H, Ar-*H*), 8.20 (s, 1H, azomethine proton), 9.30, 9.68, 10.05 (3br, 3H, 3NH, D_2O exchangeable); Its MS (*m*/*z*), 484 (M^+, 9.6%), 483 (M^+ − 1, 11.5%); $C_{26}H_{24}N_6O_4$ (484.51); Requires (Found): C, 64.45 (64.40); H, 4.99 (4.90); N, 17.35 (17.40).

*4.1.6.2. 2-[N′-(4-Chlorophenylmethylen)-hydrazone]-8,8-dimethyl-5-(4-nitrophenyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**9b**).* It was obtained from compound **2c** (3.96 g, 0.01 mol) and 4-chlorobenzaldehyde (1.40 g, 0.01 mol), crystallized from acetic acid, as yellow powder in a 70% yield, mp 330–331 °C; IR (cm^{-1}, υ): 3320 (br, NH's), 1710, 1689 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.92 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 2.10 (m, 4H, $2CH_2$), 5.04 (s, 1H, C5-*H*), 7.45 (d, J = 8.1 *Hz*, 2H, Ar-*H*), 7.80 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 7.96 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.12 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.26 (s, 1H, azomethine proton), 8.99, 9.60, 10.85 (3br, 3H, 3NH, D_2O exchangeable); Its MS (*m*/*z*), 518 (M^+, 66%), 519 (M^+ + 1, 19%), 520 (M^+ + 2, 22%); $C_{26}H_{23}ClN_6O_4$ (518.94); Requires (Found): C, 60.17 (60.25); H, 4.47 (4.50); N, 16.19 (16.12).

*4.1.6.3. 2-[N′-(4-methoxyphenylmethylen)-hydrazone]-8,8-dimethyl-5-(4-nitrophenyl)-5,8,9, 10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**9c**).* It was obtained from compound **2c** (3.96 g, 0.01 mol) and 4-methoxybenzaldehyde (1.36 g, 0.01 mol), crystallized from acetic acid, as yellow powder in a 43% yield, mp 319–320 °C; IR (cm^{-1}, υ): 3328 (br, NH's), 1714, 1683 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.89 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.89 (d, J = 17.2 *Hz*, 2H, CH_2), 2.15 (d, J = 17.2 *Hz*, 2H, CH_2), 3.33 (s, 3H, OCH_3), 4.95 (s, 1H, C5-*H*), 7.49 (d, J = 7.9 *Hz*, 2H, Ar-*H*), 7.52 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 7.69 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.01 (d, J = 8.0 *Hz*, 2H, Ar-*H*), 8.36 (s, 1H, azomethine proton), 9.50, 10.23, 10.41 (3br, 3H, 3NH, D_2O exchangeable); Its MS (*m*/*z*), 514 (M^+, 23%); $C_{27}H_{26}N_6O_5$ (514.53); Requires (Found): C, 63.02 (63.10); H, 5.09 (5.01); N, 16.33 (16.39).

*4.1.7. 3-(4-Chlorophenyl)-8,8-dimethyl-5-(4-Nitrophenyl)-5,8,9,10-tetrahydro[1,2,3] triazolo [4′,3′:1,2]pyrimido[4,5-b]quinoline-4,6-dione (**10**)*

A solution of compound **9b** (5.18 g, 0.01 mol) in acetic anhydride (30 ml) was stirred under reflux 8 h poured onto water (100 ml)

and the solid formed was collected by filtration and crystallized from ethanol, as yellow powder in a 52% yield, mp 298–299 °C; IR (cm^{-1}, υ): 3310 (br, NH's), 1705, 1690 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.91 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.98 (d, *J* = 16.9 *Hz*, 2H, CH_2), 2.25 (d, *J* = 16.9 *Hz*, 2H, CH_2), 5.01 (s, 1H, C5-*H*), 7.45 (2d, overlap, *J* = 8.2 *Hz*, 4H, Ar-*H*), 7.93 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.08 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 9.54, 11.48 (2br, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 27.71, 29.95 ($2CH_3$), 33.06, 39.83 (C-5 + C-8), 41.09, 50.99 ($2CH_2$), 93.77, 109.72, 123.98, 129.47, 129.72, 130.09, 134.11, 135.03, 14.45, 146.44 (Ar-16C), 154.34, 155.77 (2C=N), 162.60, 194.83 (2C=O); Its MS (*m*/*z*), 516 (M^+, 29%), 517 (M^+ + 1, 6%), 518 (M^+ + 2, 9%); $C_{26}H_{21}ClN_6O_4$ (516.93); Requires (Found): C, 60.41 (60.37); H, 4.09 (3.99); N, 16.26 (16.30).

4.1.8. 3,8,8-Trimethyl-2-methylthio-5-(4-nitrophenyl)-5,8,9,10-tetrahydro-pyrimido[4,5-b] quinoline-4,6-dione (**11**)

To a warm ethoxide solution (prepared by dissolving (0.23 g, 0.01 mol) of sodium in 30 ml absolute ethanol) was added compound **1c** (4.12 g, 0.01 mol), the heating was continued for 30 min, the mixture was allowed to cool to room temperature and methyl iodide (17.04 g, 0.12 mol) was added. The mixture was stirred under reflux for 3 h, cooled to room temperature, and poured onto cold water (100 ml). The solid precipitated was filtered off, washed with water and dried. The compound was crystallized from ethanol, as brown powder in a 60% yield, mp 280–282 °C; IR (cm^{-1}, υ): 3290 (br, NH's), 1700, 1682 (2C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.93 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.04 (d, *J* = 17.1 *Hz*, 2H, CH_2), 2.15 (d, *J* = 17.1 *Hz*, 2H, CH_2), 2.56 (s, 3H, SCH_3), 3.26 (s, 3H, NCH_3), 5.00 (s, 1H, C5-*H*), 7.45 (d, *J* = 7.9 *Hz*, 2H, Ar-*H*), 8.05 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 9.92 (br, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO-d_6) (δ, ppm) δ 15.38 (SCH_3), 27.75, 29.78, 30.73 ($3CH_3$), 33.11, 39.84 (C-5 + C-8), 41.09, 50.96 ($2CH_2$), 96.94, 109.56, 124.02, 129.93, 146.63, 151.39, 152.59, 154.87 (Ar-10C), 161.16 (C=N), 162.78, 194.86 (2C=O); Its MS (*m*/*z*), 426 (M^+, 11.6%), 427 (M^+ + 1, 3.6%); $C_{21}H_{22}N_4O_4S$ (426.49); Requires (Found): C, 59.14 (59.18); H, 5.20 (5.19); N, 13.14 (13.10).

4.1.9. 6-Chloro-3,8,8-trimethyl-2-methylthio-5-(4-nitrophenyl)-5,8,9,10-tetrahydro-pyrimido[4,5-b]quinoline-4,6-dione (**12**)

A mixture of compound **10** (4.26 g 0.01 mol) in phosphorus oxychloride (20 ml) was heated under reflux for 12 h. The solution was cooled and poured into ice-water (100 ml). The solid was filtered off, washed several times with water and dried. The compound was crystallized from ethanol, as pale brown powder in a 80% yield, mp 301–302 °C; IR (cm^{-1}, υ): 3278 (br, NH's), 1680 (C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.92 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.89 (d, *J* = 17.2 *Hz*, 2H, CH_2), 2.05 (d, *J* = 17.2 *Hz*, 2H, CH_2), 2.58 (s, 3H, SCH_3), 3.30 (s, 3H, NCH_3), 4.95 (s, 1H, C5-*H*), 5.77 (s, 1H, C7-*H*), 7.47 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 8.02 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 9.90 (br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 14.81 (SCH_3), 16.80, 20.82, 22.14 ($3CH_3$), 39.84, 41.10 (C-5 + C-8), 42.26 (CH_2), 110.72, 112.76, 124.83, 128.24, 130.83, 131.09, 134.42, 147.62 (Ar-12C), 162.19 (C=N), 168.26 (C=O); Its MS (*m*/*z*), 444 (M^+, 9.9%), 445 (M^+ + 1, 2.6%); $C_{21}H_{21}ClN_4O_3S$ (444.94); Requires (Found): C, 56.69 (56.66); H, 4.76 (4.78); N, 12.59 (12.51).

4.1.10. 6-Chloro-8,8-dimethyl-2-methylthio-5-(4-nitrophenyl)-5,8,9,10-tetrahydropyrimido [4,5-b]quinoline-4,6-dione (**13**)

A mixture of compound **1c** (4.12 g, 0.01) in phosphorus oxychloride (20 ml) was heated under reflux for 2 h. The solution was cooled and poured into ice-water (100 ml). The solid was filtered off, washed several times with water and dried. The compound was crystallized from ethanol, as pale yellow powder in a 85% yield, mp 325–326 °C; IR (cm^{-1}, υ): 3319 (br, NH's), 1675 (C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.83 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.98 (d, *J* = 16.9 *Hz*, 2H, CH_2), 2.15 (d, *J* = 17.0 *Hz*, 2H, CH_2), 2.55 (s, 3H, SCH_3), 4.93 (s, 1H, C5-*H*), 5.56 (s, 1H, C7-*H*), 7.40 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 8.04 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 9.90, 10.75 (2br, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 14.54 (SCH_3), 27.74, 29.71 ($2CH_3$), 33.12, 41.05 (C-5 + C-8), 50.96 (CH_2), 110.34, 124.09, 130.25, 146.62, 152.29, 152.68, 154.88, 157.25 (Ar-12C), 159.73 (C=N), 170.91 (C=O); Its MS (*m*/*z*), 308 (M^+–$C_6H_4NO_2$, 100%), 309 (M^+ + 1-$C_6H_4NO_2$, 18%), 310 (M^+ + 2-$C_6H_4NO_2$, 35%); $C_{20}H_{19}ClN_4O_3S$ (430.91); Requires (Found): C, 55.75 (55.70); H, 4.44 (4.49); N, 12.99 (12.91).

4.1.11. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(2′,3′,5′-tri-O-acetyl-β-D-arabino-furanosyl)-5,8,9,10-tetrahydropyrimido [4,5-b]quinoline-4,6-dione (**16**)

To a solution of **1c** (4.12 g, 0.01 mol) in aqueous potassium hydroxide (0.56 g, 0.01 mol) in distilled water (5 ml) was added a solution of 2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl bromide **14** (4.17 g, 0.011 mol) in acetone (30 ml). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure at 40 °C, and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from diethyl ether as pale yellow powder in a 64% yield, mp 313–314 °C; IR (cm^{-1}, υ): 3333 (br, NH), 1710–1675 (5C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.90 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 2.05 (m, 4H, $2CH_2$), 2.12–2.33 (3s, 9H, $3COCH_3$), 2.65 (s, 3H, SCH_3), 3.50 (m, 2H, H-5′, H-5″), 3.68 (m, 2H, H-4′, H-3′), 4.20 (m, 1H, H-2′), 5.02 (s, 1H, C5-*H*), 5.99 (d, $J_{1'-2'}$ = 10.2 *Hz*, 1H, H-1′), 7.48 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 8.05 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 11.02 (br, 1H, NH, D_2O exchangeable); $C_{31}H_{34}N_4O_{11}S$ (670.67); Requires (Found): C, 55.51 (55.50); H, 5.11 (5.08); N, 8.35 (8.30).

4.1.12. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(2′,3′,4′,6′-tetra-O-acetyl-β-D-gluco- or galactopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**17a**,**b**)

General procedure. To a solution of **1c** (4.12 g, 0.01 mol) in aqueous potassium hydroxide (0.56 g, 0.01 mol) in distilled water (5 ml) was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or galactopyranosyl bromide **15a**,**b** (0.011 mol) in acetone (30 ml). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure at 40 °C, and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from diethyl ether.

4.1.12.1. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(2,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**17a**).

It was obtained from compound **1c** (4.12 g, 0.01 mol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **15a** (4.5 g, 0.011 mol), crystallized from diethyl ether, as pale yellow powder in a 57% yield, mp 297–298 °C; IR (cm^{-1}, υ): 3324 (br, NH), 1718–1667 (6C=O); ^1H NMR (DMSO-d_6) (δ, ppm) δ 0.85 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.86 (d, *J* = 17.1 *Hz*, 2H, CH_2), 1.94 (d, *J* = 17.1 *Hz*, 2H, CH_2), 2.02–2.15 (4s, 12H, $4COCH_3$), 2.43 (s, 3H, SCH_3), 3.71 (m, 2H, H-6′, H-6″), 3.86 (m, 1H, H-5′), 3.98 (m, 2H, H-4′, H-3′), 5.05 (s, 1H, C5-*H*), 5.34 (m, 1H, H-2′), 6.08 (d, $J_{1'-2'}$ = 10.9 *Hz*, 1H, H-1′), 7.29 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 7.98 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 10.44 (br, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) (δ, ppm) δ 14.08 (SCH_3), 20.81–20.99 ($4COCH_3$), 27.18, 29.37 ($2CH_3$), 32.70, 39.94 (C-5 + C-8), 39.95, 50.38 ($2CH_2$), 61.98 (C6′), 68.67 (C4′), 70.32 (C2′), 71.03 (C3′), 71.18 (C5′), 71.52 (C1′), 93.37, 94.45, 96.51, 97.26, 109.50, 123.62, 123.96, 129.11, 129.63, 146.37, 152.31, 156.65, 164.26, (Ar-10C), 164.84 (C=N), 169.35, 170.09, 170.31, 170.32, 170.45, 194.36 (6C=O); $C_{34}H_{38}N_4O_{13}S$ (742.70); Requires (Found): C, 54.98 (55.00); H, 5.15 (5.18); N, 7.54 (7.50).

*4.1.12.2. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**17b**).* It was obtained from compound **1c** (4.12 g, 0.01 mol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **15b** (4.5 g, 0.011 mol), crystallized from diethyl ether, as pale yellow powder in a 59% yield, mp 311–312 °C; IR (cm^{-1}, υ): 3350 (br, NH), 1705–1669 (6C=O); ^{1}HNMR (DMSO-d_6) (δ, ppm) δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 2.01 (m, 4H, $2CH_2$), 2.08–2.28 (4s, 12H, 4COCH_3), 2.39 (s, 3H, SCH_3), 3.88 (m, 2H, H-6′, H-6″), 4.18 (m, 1H, H-5′), 4.40 (m, 2H, H-4′, H-3′), 5.00 (s, 1H, C5-*H*), 5.27 (m, 1H, H-2′), 6.08 (d, $J_{1'-2'}$ = 9.9 *Hz*, 1H, H-1′), 7.44 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 8.08 (d, *J* = 8.1 *Hz*, 2H, Ar-*H*), 10.52 (br, 1H, NH, D_2O exchangeable); $C_{34}H_{38}N_4O_{13}S$ (742.70); Requires (Found): C, 54.98 (55.05); H, 5.15 (5.10); N, 7.54 (7.48).

*4.1.13. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(β-D-arabinopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**18**)*

Dry gaseous ammonia was passed through a solution of protected arabinosides **17** (0.5 g) in dry methanol (20 ml) at room temperature for 10 min. The mixture was stirred overnight (followed by TLC). The resulting mixture was then evaporated under reduce pressure to afford a solid residue that was crystallized from diethyl ether as pale yellow in a 42% yield, mp 287–289 °C; IR (cm^{-1}, υ): 3339–3287 (br, OH, NH), 1711, 1685 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.82 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 2.00 (m, 4H, $2CH_2$), 2.68 (s, 3H, SCH_3), 3.50 (m, 2H, H-5′, H-5″), 3.74 (m, 2H, H-4′, H-3′), 4.28 (m, 1H, H-2′), 4.63 (m, 1H, OH), 4.86 (t, *J* = 4.6 Hz, 1H, OH), 5.02 (s, 1H, C5-*H*), 5.13 (m, 1H, OH), 6.10 (d, $J_{1'-2'}$ = 10.1 *Hz*, 1H, H-1′), 7.49 (d, *J* = 7.9 *Hz*, 2H, Ar-*H*), 8.10 (d, *J* = 7.9 *Hz*, 2H, Ar-*H*), 10.79 (br, 1H, NH, D_2O exchangeable); $C_{25}H_{28}N_4O_8S$ (544.58); Requires (Found): C, 55.14 (55.20); H, 5.18 (5.08); N, 10.29 (10.30).

*4.1.14. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(β-D-gluco- or galactopyranosyl)-5,8,9,10-tetrahydro-pyrimido[4,5-b] quinoline-4,6-dione (**19a**,**b**)*

General procedure. Dry gaseous ammonia was passed through a solution of protected glycosides **18a**,**b** (0.5 gm) in dry methanol (20 ml) at room temperature for 10 min. The mixture was stirred overnight (followed by TLC). The resulting mixture was then evaporated under reduce pressure to afford a solid residue that was crystallized from appropriate solvent, as pale yellow.

*4.1.14.1. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(β-D-glucopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**19a**).* It was obtained from glucosides **18a** and crystallized from diethyl ether, as pale yellow powder in a 58% yield, mp 294–295 °C; IR (cm^{-1}, υ): 3410–3358 (br, OH, NH), 1705, 1691 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.95 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.98–2.15 (m, 4H, $2CH_2$), 2.59 (s, 3H, SCH_3), 3.25 (m, 2H, H-6′, H-6″), 3.47 (m, 1H, H-5′), 3.65 (m, 2H, H-4′, H-3′), 4.21 (m, 1H, H-2′), 4.60 (m, 1H, OH), 4.86 (t, *J* = 4.8 Hz, 1H, OH), 5.08 (s, 1H, C5-*H*), 5.13 (m, 2H, 2OH), 6.10 (d, $J_{1'-2'}$ = 10.8 *Hz*, 1H, H-1′), 7.41 (d, *J* = 8.2 *Hz*, 2H, Ar-*H*), 8.08 (d, 2H, *J* = 8.1 *Hz*, Ar-*H*), 11.0 (br, 1H, NH, D_2O exchangeable); $C_{26}H_{30}N_4O_9S$ (574.60); Requires (Found): C, 54.35 (54.30); H, 5.26 (5.28); N, 9.75 (9.80).

*4.1.14.2. 8,8-Dimethyl-2-methylthio-5-(4-nitrophenyl)-10-(β-D-galactopyranosyl)-5,8,9,10-tetrahydropyrimido[4,5-b]quinoline-4,6-dione (**19b**).* It was obtained from galactosides **18b** and crystallized from diethyl ether, as pale yellow powder in a 58% yield, mp 301–302 °C; IR (cm^{-1}, υ): 3395–3318 (br, OH, NH), 1718, 1668 (2C=O); ^{1}H NMR (DMSO-d_6) (δ, ppm) δ 0.90 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.89–2.11 (m, 4H, $2CH_2$), 2.62 (s, 3H, SCH_3), 3.30 (m, 2H, H-6′, H-6″), 3.39 (m, 1H, H-5′), 3.74 (m, 2H, H-4′, H-3′), 4.09 (m, 1H, H-2′), 4.56 (m, 1H, OH), 4.79 (t, 1H, *J* = 5.0 Hz, OH), 4.98 (s, 1H, C5-*H*), 5.19 (m, 2H, 2OH), 6.11 (d, $J_{1'-2'}$ = 10.9 *Hz*, 1H, H-1′), 7.49 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 7.98 (d, *J* = 8.0 *Hz*, 2H, Ar-*H*), 10.95 (br, 1H, NH, D_2O exchangeable); $C_{26}H_{30}N_4O_9S$ (574.60); Requires (Found): C, 54.35 (54.30); H, 5.26 (5.28); N, 9.75 (9.80).

4.2. Pharmacological screening

4.2.1. Antimicrobial screening

The bacterial isolates representing Gram-negative and Gram-positive bacteria were recovered on Nutrient and MacConkey agar. The two fungal isolated on Sabouraud dextrose agar (Oxoid). They are isolated from clinical samples and identified to the species level according to different AOI systems (Biomerilux). The selected compounds were tested *in vitro* using the agar disk diffusion method taking Nalidixic acid [38] and Nystatin [39] as reference drugs for bacteria and fungi, respectively. The antimicrobial potentialities of the tested compounds were estimated by placing pre-sterilized filter paper disks (5 mm in diameter) impregnated with 50 μg/disk using dimethylsulfoxide (DMSO) as solvent which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24–28 h incubation at 37 °C for bacteria and at 28 °C after 5 days for fungi. The minimal inhibitory concentration (MIC) determination method of the biologically active compounds (Table 2) was applied using different concentrations per disk against G (−ve) and G (+ve) bacteria, and fungi. Reference Nystatin and Nalidixic acid disks were supplied by Pasteur laboratory in Egypt of concentration 100 U and 30 μg, respectively.

4.2.2. Antitumor screening

4.2.2.1. Animals. Male Swiss albino mice (body weight 20 ± 2 g). They were kept for a week under environmentally controlled conditions (constant temperature 25–27 °C, with 12 h light/dark cycle) for one week prior to starting the experiments, and they were provided with tap water and commercial diets.

4.2.2.2. Cell line. Human liver carcinoma cell line (HEPG2) and Human cervix carcinoma cell line (HELA). The cells were maintained by intraperitoneal inoculation of 1×10^6 viable cells in mice.

4.2.2.3. Ascites tumor model. Animals were divided into groups of 10 animals in each group. All the animals were injected i.p. with 1×10^6 viable (HEPG2) or (HELA) cells. After 24 h of tumor inoculation the prepared compounds were administered i.p. at dose of 10 μg/kg body weight and continued for 10 consecutive days. The group administered with vehicle alone (DMSO) was maintained as control. Cisplatine (2 mg/kg body weight, i.p., for 10 days) was used as the standard reference drug. The mortality rate was noted in each group and the percent increase in life span (ILS) was calculated as described by Ahluwalia et al. [40] and Joy et al. [41].

4.2.2.4. Assay of acute toxicity. The acute toxicity of the prepared compounds was determined *in vivo* according to Prieur et al. [42] and Ghosh [43]. Adult Swiss albino mice fasted for 12 h, were randomly divided into groups of 10 per group. Each group was separately administrated once with gradual doses of the compounds intraperitoneal (i.p.) in a value of 1 ml/kg body weight. Control animals received the vehicle alone. The fasted mice in both test and control groups were then provided with food and water immediately after the administration. Mortality of the animals was observed up to two week post-treatment. LD_{50} (the median lethal doses) of each extract was determined (the dose resulted in 50% mortality of the animals) (Table 4).

4.2.2.5. Statistical analysis. Values are recorded as mean ± SE. The data were analyzed by Student's *t*-test; differences below the 0.5 level ($P < 0.05$) was considered as statistically significant.

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