Synthetic Approach to Some New Annulated 1,2,4-Triazine Skeletons with Antimicrobial and Cytotoxic Activities

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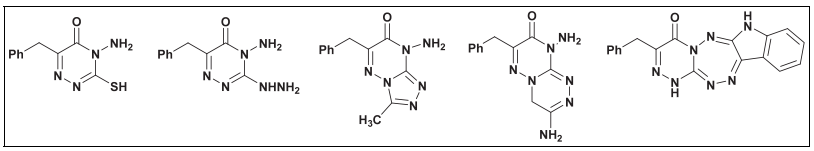

The synthesis of some triazolotriazine, tetrazolotriazine, triazinotetrazine, and triazinotetrazepinoindole by the reaction of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) with different reagents is described. The synthesized compounds were screened for their antimicrobial activity using the minimum inhibition concentration method by serial dilution technique. Two of these compounds showed higher activity than that of standard drug, and they were further evaluated for their cytotoxic activities against human cancer cells (MCF-7, HCT116, and HepG2), which indicate strong effect on these cancer cell lines.

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INTRODUCTION

In recent years, triazine-containing compounds have become potential targets for bioactive compounds discovery [1]. The 1,2,4-triazine skeleton is frequently used as a pharmacophore for the modification of known pharmaceuticals [1]. Various substituted 1,2,4-triazinone derivatives have a great importance as biological agents in medicinal and agricultural fields [1–5]. Furthermore, significant activities have been focused towards this specific substances, particularly, 4-amino-1,2,4-triazin-5-one derivatives that have predominate interest because of the herbicidal [6,7], antimicrobial [8–10], anti-HIV [11], and anticancer activities [12,13]. 1,2,4-Triazole is an important template that is connected with several biological activities as antibacterial, anticholinergic, analgesic, and antiasthmatic [14]. Likewise, triazoles are utilized in several antifungal medicines as well as fungicides [14–17]. There is considerable interest in the chemistry of heterocyclic thiones, because they are ambidextrous or multifunctional donors with sulfur or nitrogen atom. Because of the previous observations, the development of new annulated triazine systems containing 1,2,4-triazolo, triazolo, tetrazine, or tetrazepino moiety is necessary. Accordingly, these compounds were synthesized via heterocyclization reactions of trifunctional 1,2,4-triazinone derivative with different reagents. Besides, the new synthesized compounds were screened for their biological activity.

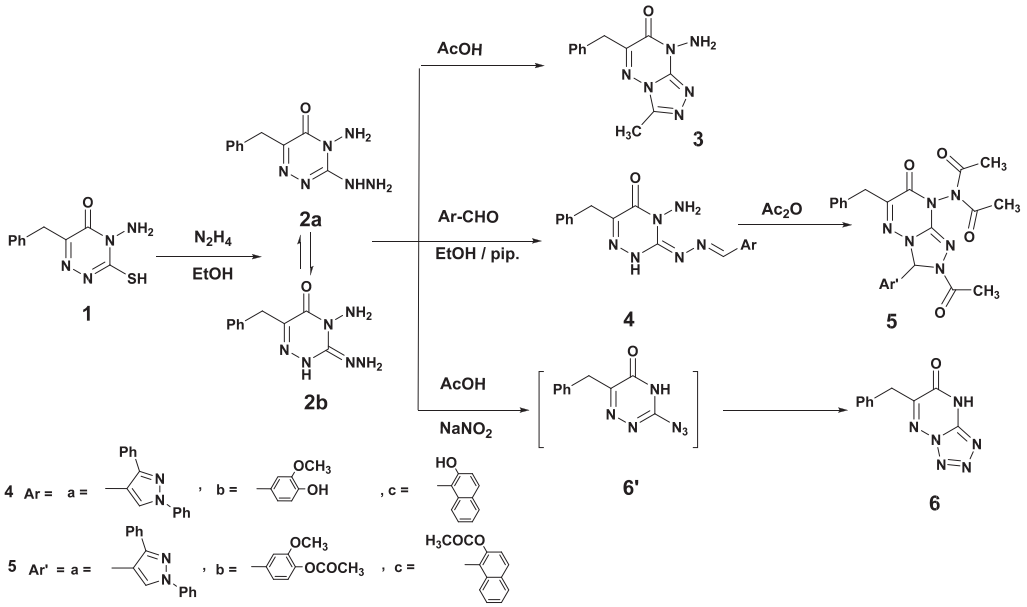
RESULTS AND DISCUSSION

Chemistry. The bridgehead nitrogen heterocyclic compounds were synthesized as depicted in Schemes 1–3. Thus, 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) has three competing nucleophilic sites: a primary amino group of the endocyclic hydrazinyl group (–N–NH_2), primary amino group of the exocyclic hydrazinyl group (–NH–NH_2), and endocyclic secondary amine (═N–NH–), which can be used as a building block unit for the synthesis of the target bridgehead nitrogen heterocyclic systems either at both the endocyclic primary amine and exocyclic amino group of hydrazinyl group to produce fused [6:6] ring size skeleton (isomer **2a**) or at both the endocyclic secondary amine and exocyclic amino group of hydrazinyl group to produce fused [6:5] ring size skeleton (isomer **2b**). Thus, cyclocondensation of **2** can be followed either pathway **A** or pathway **B** according to similar reactions previously stated [18] (Fig. 1).

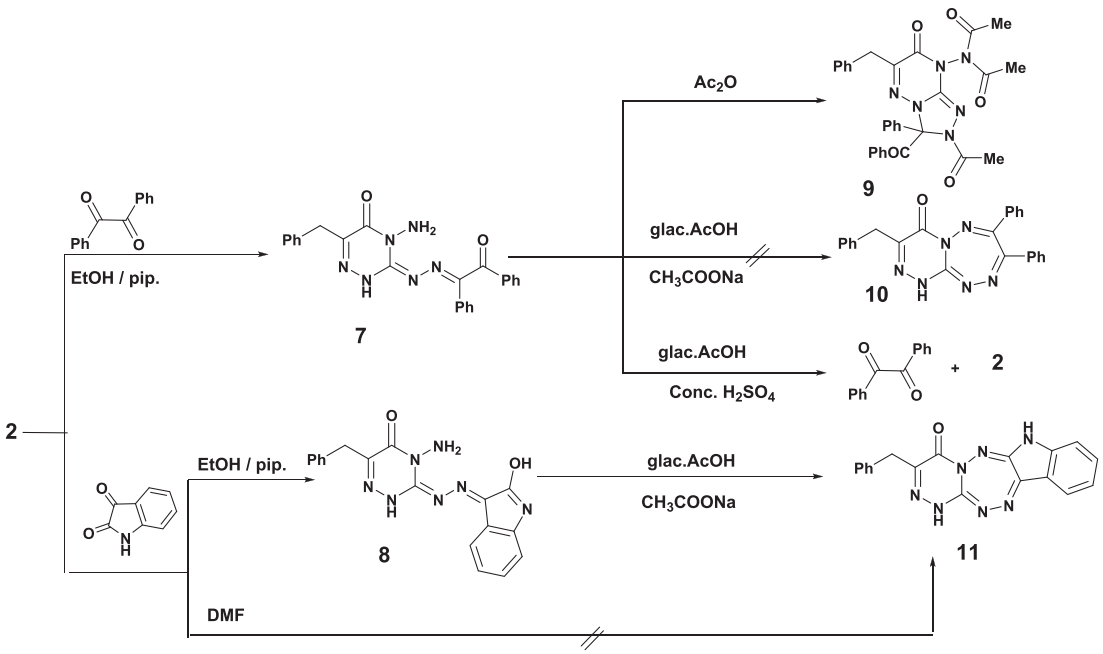
Compound **2** was obtained by reacting 4-amino 6-benzyl-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1**) with hydrazine hydrate in ethanol as a modified method for that reported in the literature [19,20]. The mass spectrum of compound **2** indicates a molecular ion peak at *m/z* = 232 (M^+ 25.3), corresponding to the molecular formula $C_{10}H_{12}N_6O$ and a base peak at *m/z* = 50 (100.0%).

Treatment of **2** with acetic acid afforded the triazolo-1,2,4-triazine **3**. The reaction occurred between hydrazide

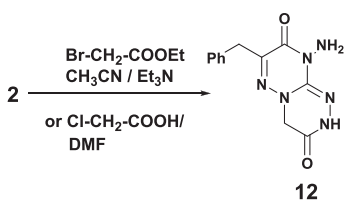
Scheme 1. Heterocyclization reaction of **2** with various reagents.



Scheme 2. Action of 1,2-diketonic compounds on the triazino hydrazine derivative **2** to form the corresponding hydrazones **7**, **8**, **9**, and **11**.



Scheme 3. Alkylation of hydrazine derivative **2** with halo compounds.



and the carbonyl group of acid to form hydrazone intermediate, which underwent a cyclization reaction between the secondary amine and the hydroxyl group of the acid function affording **3**, following pathway **A**. The constitution of **3** was deduced from its spectral data. Its ^{1}H NMR spectrum showed a singlet signal at δ = 2.49 and 6.05 ppm corresponding to CH_3 and NH_2,

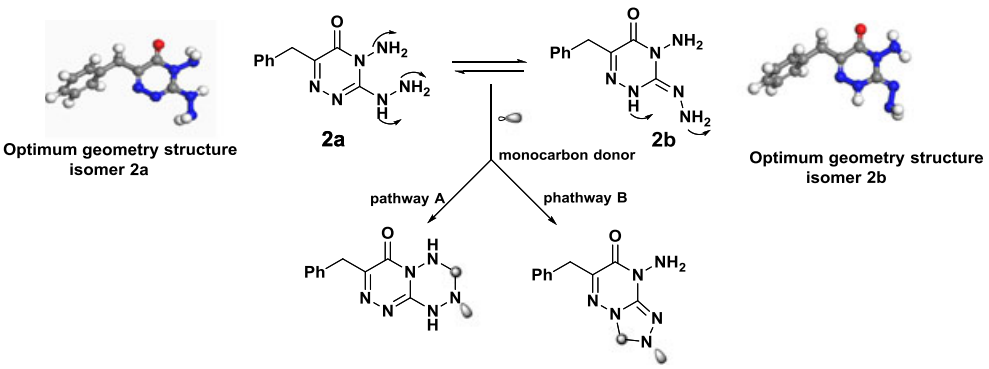


Figure 1. Pathways for the annulation reaction of compound **2**: the optimum geometrical structures of the tautomeric forms **2a** and **2b**. [Color figure can be viewed at wileyonlinelibrary.com]

respectively, and a multiplet at δ = 7.06–7.26 ppm for five aromatic protons (Scheme 1).

Additionally, condensation of **2** with a variety of one carbon donor, such as 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde, 4-hydroxy-3-methoxybenzaldehyde, and 2-hydroxy-1-naphthaldehyde, affords the corresponding Schiff bases **4a**–**c**. The structures of these compounds were based on their spectroscopic analysis. Thus, the main characteristic features for the ^{1}H NMR of compounds **4a**–**c** revealed new signals at δ = 9.28, 8.17, and 9.40 ppm for ═CH groups and at δ = 12.20, 12.23, and 12.33 ppm for NH protons, respectively (Scheme 1).

Furthermore, treatment of compound **4a**–**c** with acetic anhydride gives triacetylated heterobicyclic derivatives **5a**–**c**. The structures of these compounds were established by their spectral analyses. Thus, the ^{1}H NMR spectrum of **5a** showed the absence of ═CH and NH protons and the appearance of new singlet signals at δ = 2.07, 2.37, and 2.40 ppm corresponding to (OC–$CH_{3\text{triazole}}$) and (2–CH_3CO–) groups besides the benzylic and aromatic protons. Besides, the ^{1}H NMR spectra of **5b**,**c** showed the absence of ═CH and NH protons and the appearance of singlet signals for (CH_3COO–) protons at δ = 2.31 and 2.49 ppm, respectively. Furthermore, the structures of **5b**,**c** were supported by ^{13}C NMR, which showed the quaternary carbon at δ = 76.66 and 67.59 ppm, respectively, which indicated that the formation of the target compounds went along pathway **B** (Scheme 1).

Deamination of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) using nitrous acid in acetic acid gave the corresponding tetrazolo[5,1-*c*]1,2,4-triazine derivative (**6**) through the formation of the non-isolable azido intermediate 6′. Assignment of the structure for compound **6** was based on its spectroscopic analysis. Thus, ^{1}H NMR spectrum of **6** showed the absence of the NH and NH_2 protons of hydrazinyl group and the appearance of the new NH proton signal at δ = 12.03 ppm (Scheme 1).

Once more, 1,2-diketonic compounds were also used as two-carbon building block for the synthesis of fused heterocyclic systems. Thus, 4-amino-6-benzyl-3-((2-oxo-1,2-diphenyl-ethylidene)hydrazono)-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**7**) was prepared by refluxing **2** with benzil in ethanol in the presence of a few drops of piperidine. The ^{1}H NMR spectrum of **7** showed singlet signals at δ = 5.39 and 12.65 ppm corresponding to NH_2 and NH protons, respectively (Scheme 2).

Similarly, treating of **2** with isatine in DMF and/or absolute ethanol in the presence of few drops of piperidine afforded 4-amino-6-benzyl-3-((2-hydroxy-3*H*-indol-3-ylidene)hydrazono)-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**8**). The ^{1}H NMR spectrum of **8** showed singlet signals at δ = 6.09, 10.68, and 11.17 ppm NH_2, NH, and OH protons, respectively (Scheme 2).

Reaction of **7** with acetic anhydride afforded *N*-acetyl-*N*-(2-acetyl-3-benzoyl-6-benzyl-7-oxo-3-phenyl-2,3-dihydro-1,2,4-triazolo[4,3-*b*]1,2,4-triazin-8(7*H*)-yl)acetamide (**9**), which showed singlet signals at 1.93, 2.50, and 2.58 ppm corresponding to (–OC–$CH_{3\text{triazole}}$) and (2 CH_3–CO–) groups, respectively. Also, ^{13}C NMR spectrum of **9** showed the presence of quaternary carbon at 87.16 ppm, which indicated the formation of the target compounds via pathway **B** (Scheme 2).

Our trial to prepare 3-benzyl-7,8-diphenyl-[1,2,4]triazino[4,3-*b*][1,2,4,5]tetrazepin-4(1*H*)-one (**10**) by refluxing compound **7** in glacial acetic acid in the presence of anhydrous fused sodium acetate was unsuccessful. While refluxing compound **7** in glacial acetic acid in the presence of concentrated H_2SO_4 gave back the starting compound **2** and benzil [19] (Scheme 2), our attempt to synthesize 3-benzyl-1,7-dihydro-4*H*-1,2,4-triazino[3′,4′:3,4]1,2,4,5-tetrazepino [6,7-*b*]indol-4-one (**11**) by refluxing **2** with isatine in DMF was failed (Scheme 2).

Ring closure of **8** in boiling acetic acid in the presence of sodium acetate afforded 3-benzyl-1,7-dihydro-4*H*-1,2,4-triazino[3′,4′:3,4]1,2,4,5-tetrazepino [6,7-*b*]indol-4-

one (**11**). Its IR indicates that OH and NH_2 were disappeared. The ^1H NMR spectra show the presence of $NH_{triazine}$ and NH_{indole} protons at δ = 4.19 and 11.13, respectively. In addition, the mass spectrum of compound **11** showed an ion peak at 341 ($M^+ - 2$, 30.8) and a base peak at *m/z* = 60 (100.0%) corresponding to the molecular formula $C_{18}H_{13}N_7O$ (Scheme 2).

Alkylation of compound **2** with either ethyl bromoacetate in acetonitrile in the presence of TEA or monochloroacetic acid in DMF furnished 9-amino-7-benzyl-2,9-dihydro-8*H*-1,2,4-triazino[4,3-*b*]1,2,4-triazine-3,8(4*H*)-dione (**12**) via pathway **B** (Scheme 3). The structures of this compound were established by its spectral analysis. The IR spectrum of compounds **12** showed peaks at 3330, 3305, 3243, and 1689 cm^{-1} for NH_2, NH, and (C═O amide) groups, respectively. Its ^1H NMR spectra showed singlet signals at δ = 3.80 (s, 4H, CH_2–Ph + endocyclic CH_2), 6.03 (s, 2H, NH_2, D_2O exchangeable), and 11.94 ppm (s, 1H, NH, D_2O exchangeable).

Biological activity.

Antimicrobial activity. Compounds **1**–**12** were evaluated for their antimicrobial activity against *Bacillus subtilis* (*B. subtilis*) and *Streptococcus pneumoniae* (*S. pneumonia*) as examples of Gram-positive bacteria, against *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) as examples of Gram-negative bacteria, and against *Aspergillus fumigates* (*A. fumigatus*) and *Candida albicans* (*C. albicans*) fungal strains. The diameters (mm) of inhibition zones were determined using the standard agar diffusion assay, and minimal inhibitory concentration (MIC) was also determined and summarized in Tables 1 and 2. The amphotericin B and the ampicillin were used as references to compare the potency of the tested compounds under the same conditions.

Generally, all the compounds exhibit good antifungal activity against *A. fumigatus* and *C. albicans* fungal strains with compounds **2**, **4a**, **4b**, **7**, **8**, and **12** being the most potent. On the other hand, the rest of the compounds (e.g., **1** and **11**) showed more moderate activity against the used fungal strains (Table 2). Compound **4c** was found to be the most effective against *P. aeruginosa* and *E. coli*, the Gram-negative bacteria with inhibition zones of 18.3 and 22.6 mm, respectively, whereas compound **8** was found to be effective against both Gram-positive and

Table 2

In vitro antifungal activity of synthesized compounds **1**–**13** through the well diffusion method.

	Diameter growth of inhibition zone in mm	
Compound no.	*A. fumigatus*	*C. albicans*
1	15.7 ± 0.33	13.8 ± 0.25
2	18.7 ± 0.36	16.9 ± 0.27
3	17.6 ± 0.58	15.4 ± 0.25
4a	13.6 ± 0.25	11.7 ± 0.34
4b	18.2 ± 0.44	12.6 ± 0.42
4c	17.3 ± 0.44	16.9 ± 0.25
5a	12.6 ± 0.25	11.2 ± 0.36
5b	15.7 ± 0.36	13.3 ± 0.36
5c	16.8 ± 0.39	15.9 ± 0.44
6	15.3 ± 0.55	13.4 ± 0.35
7	20.2 ± 0.55	19.6 ± 0.33
8	16.4 ± 0.52	13.9 ± 0.39
9	17.5 ± 0.44	19.8 ± 0.63
11	12.8 ± 0.34	15.4 ± 0.53
12	23.8 ± 0.20	32.4 ± 0.30
Amphotericin B	23.7 ± 0.1	25.4 ± 0.1

Table 1

In vitro antibacterial activity of synthesized compounds **1**–**12** through the well diffusion method.

	Diameter growth of inhibition zone in mm			
	Gram-positive bacteria		Gram-negative bacteria	
Compound no.	*S. pneumonia*	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	16.9 ± 0.58	18.2 ± 0.44	NA	11.9 ± 0.63
2	12.9 ± 0.63	13.2 ± 0.58	NA	10.8 ± 0.44
3	12.3 ± 0.58	12.7 ± 0.37	NA	8.5 ± 0.37
4a	14.6 ± 0.58	14.3 ± 0.58	NA	9.4 ± 0.44
4b	17.5 ± 0.44	19.8 ± 0.63	8.5 ± 0.37	9.3 ± 0.42
4c	16.3 ± 0.55	18.3 ± 0.25	18.3 ± 0.25	22.6 ± 0.44
5a	NA	NA	NA	NA
5b	NA	NA	NA	NA
5c	16.7 ± 0.36	19.2 ± 0.27	NA	13.6 ± 0.36
6	17.5 ± 0.44	19.8 ± 0.63	NA	18.9 ± 0.25
7	13.2 ± 0.58	14.6 ± 0.58	12.3 ± 0.58	16.4 ± 0.58
8	24.6 ± 0.24	35.9 ± 0.29	18.7 ± 0.12	20.4 ± 0.32
9	11.9 ± 0.32	19.8 ± 0.63	12.8 ± 0.38	10.9 ± 0.35
11	14.1 ± 0.52	12.7 ± 0.37	11.6 ± 0.35	9.1 ± 0.37
12	13.2 ± 0.38	13.1 ± 0.31	NA	10.2 ± 0.41
Ampicillin	23.8 ± 0.20	132.4 ± 0.30	17.3 ± 0.10	19.9 ± 0.30

Gram-negative bacteria with a similar antibacterial activity to the standard ampicillin.

Minimum inhibitory concentration. Furthermore, compounds **4c** and **8** were further selected to determine the MIC employing the agar dilution method. The MIC (μg/mL) values for most active compounds **4c** and **8** are depicted in Table 3. It was found that compounds **4c** and **8** exhibit variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains and also against antifungal strain. Within this context, the antibacterial activity of compound **4c** was seven times higher than ampicillin with MIC of 76.34 and 37.25 μg/mL against *S. pneumonia* and *B. subtilis*, respectively. On the other hand, compound **8** was two times higher than ampicillin in the case of *S. pneumonia*

Table 3

Minimal inhibitory concentration (MIC, μg/mL) of compounds **4c** and **8**.

	MIC in (μg/mL) and zone of inhibition in mm					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
Compound no.	S. pneumonia	*B. subtilis*	*P. aeruginosa*	*E. coli*	*A. fumigatus*	*C. albicans*
4c	76.34 ± 0.25	37.25 ± 0.18	NA	28.37 ± 0.53	42.63 ± 0.82	35.24 ± 0.47
8	25.12 ± 0.33	22.41 ± 0.63	NA	34.25 ± 0.23	NA	NA
Ampicillin	10.58 ± 0.25	5.29 ± 0.27	17.96 ± 0.30	16.24 ± 0.37	—	—
Amphotericin B	—	—	—	—	11.24 ± 0.34	12.68 ± 0.21

Table 4

In vitro cytotoxic activities of **4c** and **8** (MCF-7) cell line.

	% Inhibitions					
	Concentration (μg/mL)					
Compound no.	50	25	12.5	6.25	3.125	1.56
4c	2.87	5.74	13.06	27.19	53.42	67.58
8	3.75	10.83	25.11	46.88	57.03	68.15
Doxorubicin	67.54	77.09	81.29	87.76	92.77	96.58

Table 5

In vitro cytotoxic activities of **4c** and **8** (HepG2) cell line.

	% Inhibitions					
	Concentration (μg/mL)					
Compound no.	50	25	12.5	6.25	3.125	1.56
4c	5.71	10.83	24.72	38.17	59.03	82.22
8	3.44	12.81	29.38	43.24	65.18	76.06
Doxorubicin	51.75	69.68	78.97	83.10	85.71	89.05

Table 6

In vitro cytotoxic activities of **4c** and **8** (HCT116) cell line.

	% Inhibitions					
	Concentration (μg/mL)					
Compound no.	50	25	12.5	6.25	3.125	1.56
4c	4.92	12.74	25.33	52.61	62.86	73.43
8	7.42	13.33	26.41	58.74	67.19	76.22
Doxorubicin	66.87	76.35	81.84	84.74	91.27	94.62

with MIC of 25.12 μg/mL and four times higher than ampicillin in the case of *B. subtilis* with MIC of 22.41 μg/mL.

In vitro cytotoxic activities. Encouraged by the promising antimicrobial activities exhibited by compounds **4c** and **8**, their cytotoxicity were therefore assessed in colon carcinoma (HCT116), breast adenocarcinoma (MCF-7), and hepatocellular carcinoma (HepG2) cell lines employing the crystal violet assay using doxorubicin as a standard anticancer drug in Tables 4–6. The IC_{50} values are calculated from the dose–response curves and are shown in Table 7.

In general, compounds **4c** and **8** showed good anticancer activity with more pronounced cytotoxicity in the case of MCF-7 compared with HCT116 and HepG2 cells in Figures 2–4. Furthermore, compound **8** was generally more cytotoxic than **4c**. This may be attributed to the presence of indolyl moiety in the case of compound **8**.

In general, compounds **4c** and **8** showed good cytotoxicity against MCF-7, HCT116, and HepG2 cells.

Molecular modeling.

Geometry optimization. Geometry optimization of the molecular structures for the different compounds was carried out using the density functional theory via cluster calculations using DMOL3 program [21] in Materials Studio package [22] in order to investigate their stability relative to each other. The values resulted for the main quantum parameters are summarized in Table 8.

The orientation of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) exists in two tautomeric structures **2a** and **2b**. The results of binding energies calculations showed that **2b** isomer has a binding energy value with 0.18418 eV more negative than that of isomer **2a**. So the isomer **2b** is more stable than the isomer **2a**. Furthermore, the difference between the frontier orbitals ($E_{HOMO} - E_{LUMO}$) indicates that the **2b** is more chemically stable than **2a**.

Table 7

Cytotoxic activity of compounds **4c** and **8** against MCF-7, HepG2, and HCT116 human tumor cells.

Compound no.	IC_{50} (μg/mL)		
	MCF-7	HepG2	HCT116
4c	11.9	19.6	23.4
8	10.8	16.4	16.3
Doxorubicin	0.44	1.2	0.46

IC_{50} (μg/mL): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and above 100 (noncytotoxic).

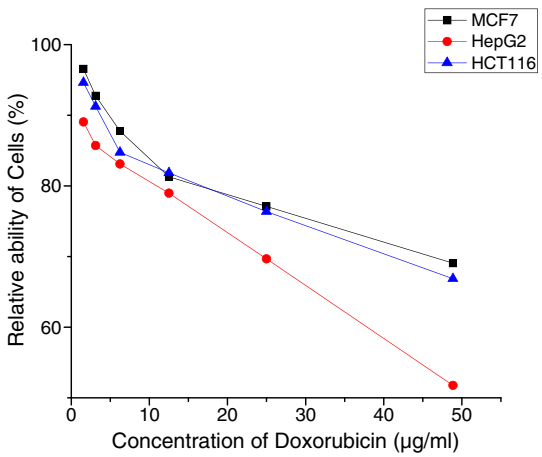


Figure 2. Antibacterial activity of compound doxorubcin against MCF-7, HepG2, and HCT116. [Color figure can be viewed at wileyonlinelibrary.com]

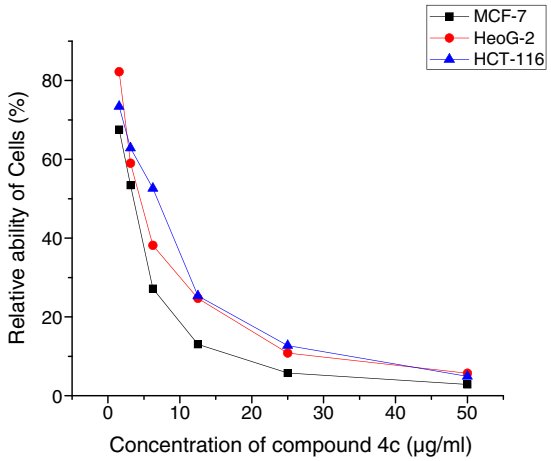


Figure 3. Antibacterial activity of compound **4c** against MCF-7, HepG2, and HCT116. [Color figure can be viewed at wileyonlinelibrary.com]

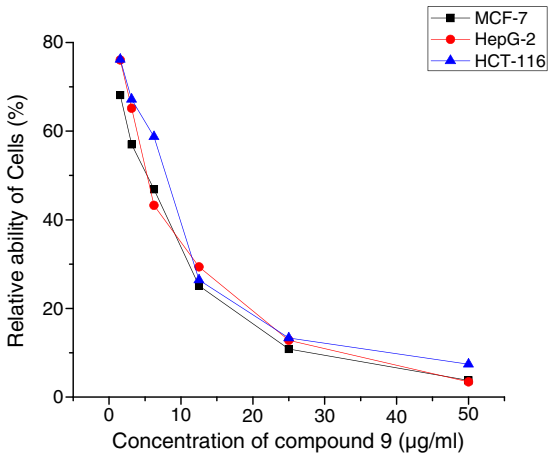


Figure 4. Antibacterial activity of compound **9** against MCF-7, HepG2, and HCT116. [Color figure can be viewed at wileyonlinelibrary.com]

Table 8
Total energies, binding energies, and frontier orbital energies of compound **30**.

Compound no.	Binding energy (eV)	Total energy (eV)	HOMO (eV)	LUMO (eV)	$E_{(HOMO\ -\ LUMO)}$
30a	−134.45184	−21558.27581	−5.263	−2.154	−3.109
30b	−134.63602	−21558.45358	−4.376	−2.249	−2.127

CONCLUSIONS

New ring junction nitrogen compounds as triazolotriazine, tetrazolotriazine, triazinotetrazine, and triazinotetrazepino were designed and synthesized by the reaction of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) with different reagents. SAR estimations and antimicrobial and cytotoxic activities for ring junction nitrogen compounds were evaluated and were found to exhibit considerable antimicrobial and cytotoxic effects.

EXPERIMENTAL

Instruments. All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electronic melting point apparatus. Elemental analyses have been carried out at Micro Analytical Center, Faculty of Science, Cairo University. IR spectra have been recorded (KBr), (ύ cm^{-1}) on a Mattson 5000 FTIR Spectrophotometer at Micro Analytical Center, Faculty of Science, Mansoura University. The ^1H NMR and ^{13}C NMR spectra have been measured on a Varian Spectrophotometer at 300, 75 MHz, respectively. TMS was used as an internal reference and $CDCl_3$ and DMSO-d_6 as solvent at Chemistry Department, Faculty of Science, Cairo University. The chemical shifts (δ) have been reported in parts per million and have been referenced to the residual solvent peak. Mass spectra have been recorded on Kratos (70 eV) MS equipment and/or a Varian MAT 311A Spectrometer, at Micro Analytical Center, Faculty of Science, Cairo University. Reaction mixtures have been monitored by thin-layer chromatography using EM science silica gel-coated plates with visualization by irradiation with ultraviolet lamp. Biological testing has been carried out at the Regional Center for Mycology and Biotechnology, Antimicrobial Activity Unit, Al-Azhar University, Cario, Egypt.

Synthesis of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4H)-one (2). A mixture of triazine **1** (0.23 g, 1 mmol) and hydrazine hydrate (0.2 mL, 4 mmol) in ethanol (50 mL) was refluxed for 6 h, cooled at room temperature, and the formed precipitate was separated by filtration and recrystallized to afford **2**.

White needles (0.84 g, 85%); mp 265–266°C (lit. mp °C 254–255 [20]); [ethanol]; R_f = 0.14 [pet. ether (60:80)/ethyl acetate (4:1)]; IR (KBr): ν/cm^{-1} = 3330, 3283, 3226 ($2NH_2$), 3184 (NH), 2910 (CH_2), 1685 (C═O amide), and 1660 (C═C); MS (EI, 70 eV) *m/z* (%) = 232 (M^+ 25.3), 216 (3.8), 185 (1.6), 186 (5.8), 117 (24.1), 91 (96.7), 76 (67.4), 53 (27.6), 50 (100.0). *Anal*. Calcd for $C_{10}H_{12}N_6O$ (232.52): C, 51.72; H, 5.21; N, 36.19%. Found: C, 51.70; H, 5.24; N, 36.22%.

8-Amino-6-benzyl-3-methyl-1,2,4-triazolo[4,3-b]1,2,4-triazin-7(8H)-one (3). A mixture of hydrazinyl **2** (0.5 g, 2.16 mmol) and acetic acid (15 mL) was refluxed for 2 h. The precipitate which formed on hot was separated by filtration and recrystallized to afford **3**.

Yellow flakes (0.42 g, 76%); mp 295–296°C; [DMF]; R_f = 0.49 [ethyl acetate/ethanol (4:0.5)]; IR (KBr): ν/cm^{-1} = 3305, 3244 (NH_2), 2910 (CH_2), 1689 (C═O amide), and 1660 (C═C); ^1H NMR (DMSO-d_6) δ (ppm): 2.49 (s, 3H, CH_3), 3.81 (s, 2H, CH_2–Ph), 6.05 (s, 2H, NH_2), 7.19–7.26 (m, 5H, Ar–H); MS (EI, 70 eV) *m/z* (%) = 258 (M^+ +2, 1.0), 256 (1.0), 245 (100.0), 243 (1.0), 233 (7.8), 231(1.0), 217 (9.6), 203 (2.9), 187 (1.0), 185 (9.9), 117 (35.3), 96 (1.0), 92 (86.2), 91 (5.2), 76 (5.7), 52 (17.4), 51 (30.6), 50 (2.0). *Anal*. Calcd for $C_{12}H_{12}N_6O$ (256.27): C, 56.24; H, 4.72; N, 32.79%. Found: C, 56.27; H, 4.75; N, 32.82%.

General procedure for synthesis of Schiff bases 4a–c. To a solution of hydrazinyl **2** (0.5 g, 2.16 mmol) and the proper aldehyde (2.16 mmol) in ethanol (50 mL) piperidine (five drops) was added and heated under reflux for 3–6 h. The precipitate which formed on hot (except for 4*b* upon cooling) was separated by filtration and recrystallized to afford **4a–c**.

4-Amino-6-benzyl-3-(((1,3-diphenyl-1H-pyrazol-4-yl) methylene)hydrazono)-3,4-dihydro-1,2,4-triazin-5(2H)-one (4a). The aldehyde used was 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde.

Yellow powder (0.82 g, 82%) (3 h reflux); mp 201–203°C; [DMF]; R_f = 0.26 [pet. ether (60:80)/ethyl acetate (4:1.5)]; IR (KBr): ν/cm^{-1} = 3280, 3265 (NH_2), 3176 (NH), 2910 (CH_2), 1663 (C═O amide), and 1660 (C═C); ^1H NMR (DMSO-d_6) δ (ppm): 3.89 (s, 2H, CH_2–Ph), 5.76 (s, 2H, NH_2), 7.29–7.89 (m, 15H, Ar–H), 8.32 (s, 1H, CH–pyrazole), 9.28 (s, 1H, ═CH), 12.21 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 463(M^+ +1, 23.7), 462 (96.0), 447 (100.0), 220 (6.4), 187 (13.1), 157 (3.5), 59 (97.5). *Anal*. Calcd for $C_{26}H_{22}N_8O$ (462.52): C, 67.52; H, 4.79; N, 24.23%. Found: C, 67.55; H, 4.82; N, 24.20%.

4-Amino-6-benzyl-3-((4-hydroxy-3-methoxybenzylidene) hydrazono)-3,4-dihydro-1,2,4-triazin-5(2H)-one (4b). The aldehyde used was 4-hydroxy-3-methoxy benzaldehyde.

Yellow powder (0.67 g, 85%) (6 h reflux); mp 180–181°C; [ethanol]; R_f = 0.28 [pet. ether (60:80)/ethyl acetate (4:2)]; IR (KBr): ν/cm^{-1} = 3328 (OH), 3265, 3245 (NH_2), 3176 (NH), 2910 (CH2), 1663 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.85 (s, 2H, CH_2–Ph), 3.88 (s, 3H, O–CH_3), 5.75 (s, 2H, NH_2), 6.79–7.74 (m, 8H, Ar–H), 8.17 (s, 1H, ═CH), 9.35 (br, 1H, OH), 12.23 (br, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 367 (M^+ +1, 20.1), 366 (87.9), 351 (14.2), 218 (12.3), 217 (29.4), 150 (40.5), 136 (76.1), 117 (17.6), 116 (40.0), 91 (100.0), 77 (16.5), 76 (5.4), 51 (4.9). *Anal*. Calcd for $C_{18}H_{18}N_6O_3$ (366.38): C, 59.01; H, 4.95; N, 22.94%. Found: C, 59.03; H, 4.92; N, 22.97%.

4-Amino-6-benzyl-3-(((2-hydroxynaphthalen-1-yl)methylene) hydrazono)-3,4-dihydro-1,2,4-triazin-5(2H)-one (4c). The aldehyde used was 2-hydroxy naphthaldehyde.

Yellow powder (0.72 g, 87%) (4 h reflux); mp 228–229°C; [DMF]; R_f = 0.47 [pet. ether (60:80)/ethyl acetate (4:2)]; IR (KBr): ν/cm^{-1} = 3313 (OH), 3240, 3223 (NH_2), 3186 (NH), 2910 (CH_2), 1663 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.91 (s, 2H, CH_2–Ph), 5.87 (br s, 2H, NH_2), 7.15–8.66 (m, 11H, Ar–H), 9.40 (s, 1H, ═CH), 11.65 (br s, 1H, OH), 12.33 (br, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 387 (M^+ +1, 23.7), 386(100.0), 369 (22.2), 218 (60.2), 170 (36.7), 169 (75.6), 143 (5.2), 128 (95.4), 117 (13.0), 116 (44.8), 115 (60.9), 103 (6.2), 102 (10.7), 91 (50.4), 77 (9.2), 51 (1.9). *Anal*. Calcd for $C_{21}H_{18}N_6O_2$ (386.42): C, 65.27; H, 4.70; N, 21.75%. Found: C, 65.24; H, 4.73; N, 21.73%.

Effect of acetic anhydride on Schiff bases 5a–c. General procedure: A solution of the corresponding Schiff bases **4a–c** (2.5 mmol) in 6 mL acetic anhydride was heated under reflux for 12 h. The reaction mixture was then cooled and poured into crushed ice. The crude product that precipitated was obtained by filtration, washed with water (10 mL), and then recrystallized to afford **5a–c**.

N-Acetyl-N-(2-acetyl-6-benzyl-3-(1,3-diphenyl-1H-pyrazol-4-yl)-7-oxo-2,3-dihydro-1,2,4-triazolo[4,3-b][1,2,4-triazin-8(7H)-yl)acetamide (5a). Yellow flakes (1.2 g, 79%); mp 160–162°C; [ethyl acetate]; R_f = 0.50 [pet. ether (60–80)/ethyl acetate (4:2)]; IR (KBr): ν/cm^{-1} = 2910 (CH_2), 1648 (O═C–CH_3), 1644 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.07 (s, 3H, $CH_{3\text{triazole}}$), 2.37 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.80 (s, 2H, CH_2–Ph), 7.12–7.78 (m, 16H, Ar–H + CH–triazole), 8.15 (s, 1H, CH–pyrazole); MS (EI, 70 eV) *m/z* (%) = 589 (M^+ +1, 68.7), 588 (79.4), 488 (32.9), 487 (100.0), 455 (8.9), 403 (8.6), 401 (23.6), 360 (7.9), 317 (17.7), 298 (10.0), 274 (12.5), 256 (18.7), 241 (16.5), 231 (18.7), 221 (10.1), 218 (1.1), 124 (31.0). *Anal*. Calcd for $C_{32}H_{28}N_8O_4$ (588.63): C, 65.30; H, 4.79; N, 19.04%. Found: C, 65.27; H, 4.81; N, 19.01%.

4-(2-Acetyl-8-(N-acetylacetamido)-6-benzyl-7-oxo-2,3,7,8-tetrahydro-1,2,4-triazolo[4,3-b][1,2,4-triazin-3-yl)-2-methoxyphenyl acetate (5b). Yellow powder (1 g, 75%); mp 195–197°C; [ethyl acetate]; R_f = 0.50 [pet. ether (60–80)/ethyl acetate (4:2.5)]; IR (KBr): ν/cm^{-1} = 2915 (CH_2), 1770 (OCO–CH_3), 1647 (O═C–CH_3), 1643 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.03 (s, 3H, $CH_{3\text{triazole}}$), 2.31 (s, 3H, CH_3–COO), 2.35 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.81 (s, 2H, CH_2–Ph), 3.84 (s, 3H, O–CH_3), 7.01–7.26 (m, 9H, Ar–H + CH–triazole); ^{13}C NMR (DMSO-d_6) δ: 20.73 (1C, OCO–$\underline{C}H_3$), 20.79 (1C, O═C–$\underline{C}H_3$), 23.99 (1C, O═C–$\underline{C}H_3$), 24.92 (1C, O═C–$\underline{C}H_3$), 36.52 (1C, CH_2–Ph), 56.05 (1C, O–CH_3), 76.66 (1C, C-3`), 110.84 (1C, C_{Ar}), 119.43 (1C, CH_{Ar}), 123.12 (1C, CH_{Ar}), 127.04 (1C, CH_{Ar}), 128.58 (2C, CH_{Ar}), 128.76 (2C, C_{Ar}), 134.02 (1C, C_{Ar}), 138.56 (1C, C_{Ar}), 140.94 (1C, C_{Ar}), 143.12 (1C, C-3), 144.48 (1C, C-6), 151.52 (1C, C_{Ar}), 167.39 (2C, C═O), 168.45 (1C, COO), 168.74 (2C, C═O); MS (EI, 70 eV) *m/z* (%) = 535 (M^+ +1, 29.5), 534 (100.0), 492 (71.8), 451 (16.2), 450 (89.6), 449 (12.2), 434 (3.6), 433 (9.9), 408 (27.0), 407 (19.9), 392 (20.5), 391 (38.3), 366 (2.9), 365 (3.5), 302 (2.5), 285 (10.6), 260 (15.5), 218 (8.6), 150 (25.0), 149 (15.7), 136 (32.5), 117 (6.1), 116 (10.1), 91 (83.7), 77 (8.8), 76 (2.3), 52 (1.9), 51 (3.5), 50 (1.0). *Anal*. Calcd for $C_{26}H_{26}N_6O_7$ (534.53): C, 58.42; H, 4.90; N, 15.72%. Found: C, 58.39; H, 4.88; N, 15.70%.

1-(2-Acetyl-8-(N-acetylacetamido)-6-benzyl-7-oxo-2,3,7,8-tetrahydro-1,2,4-triazolo[4,3-b][1,2,4-triazin-3-yl)naphthalen-2-yl)acetate (5c). Yellow powder (1 g, 73%); mp 165–166°C; [ethyl acetate]; R_f = 0.30 [pet. ether (60:80)/ethyl acetate (4:3)]; IR (KBr): ν/cm^{-1} = 2915 (CH_2), 1764 (OCO–CH_3), 1651 (O═C–CH_3), 1644 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.98 (s, 3H, $CH_{3\text{triazole}}$), 2.49 (s, 3H, CH_3–COO), 2.57 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 3.71 (s, 2H, CH_2–Ph), 6.90–8.05 (m, 12H, Ar–H + CH–triazole); ^{13}C NMR (DMSO-d_6) δ: 17.83 (1C, OCO–$\underline{C}H_3$), 23.18 (1C, O═C–$\underline{C}H_3$), 25.96 (1C, O═C–$\underline{C}H_3$), 27.39 (1C, O═C–$\underline{C}H_3$), 38.20 (1C, CH_2–Ph), 67.59 (1C, C-3`), 123.32 (1C, CH_{Ar}), 123.43 (1C, C_{Ar}), 125.21 (1C, CH_{Ar}), 125.63 (1C, CH_{Ar}), 125.92 (1C, CH_{Ar}), 128.52 (2C, CH_{Ar}), 129.27 (1C, CH_{Ar}), 130.99 (2C, CH_{Ar}), 131.13 (2C, CH_{Ar}), 131.78 (1C, C_{Ar}), 134.43 (2C, CH_{Ar}), 135.05 (1C, C_{Ar}), 138.75 (1C, C_{Ar}), 147.65 (1C, C-3), 144.56 (1C, C-6), 153.96 (1C, C_{Ar}), 170.76 (1C, O–$\underline{C}O$–CH_3), 172.48 (2C, 2O═$\underline{C}$–CH_3); MS (EI, 70 eV) *m/z* (%) = 554 (M^+ 18.4), 512 (14.0), 511 (4.6), 496 (26.7), 468 (15.0), 454 (1.2), 452 (10.9), 411 (35.2), 369 (33.9), 325 (11.6), 283 (14.2), 225 (1.4), 223 (12.4), 186 (4.3), 185 (22.5), 171 (13.2), 170 (41.4), 169 (100.0), 117 (7.3), 116 (14.7), 115 (31.9), 91 (98.3), 77 (16.8), 52 (1.0), 51 (5.4), 50 (4.3). *Anal*. Calcd for

$C_{29}H_{26}N_6O_6$ (554. 56): C, 62.81; H, 4.73; N, 15.15%. Found: C, 62.83; H, 4.70; N, 15.12%.

6-Benzyltetrazolo[1,5-b]1,2,4-triazin-7(8H)-one (6). To a solution of hydrazinyl **2** (0.5 g, 2.16 mmol) in acetic acid (22 mL) was added a solution of sodium nitrite (1.3 g, 18.8 mmol) in 1.5 mL water dropwise with stirring and cooling at 0°C over a period of 1 h, and the reaction mixture was then kept in the refrigerator overnight. The reaction mixture was diluted with ice water, and the formed precipitate was collected, dried at room temperature, and recrystallized to afford **6**.

White needle (0.33 g, 68%); mp 233–235°C; [ethanol]; R_f = 0.47 [pet. ether (60:80)/ethyl acetate (4:3.5)]; IR (KBr): ν/cm^{-1} = 3165 (NH), 2915 (CH_2), 1653 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.81 (s, 2H, CH_2–Ph), 7.19–7.28 (m, 5H, Ar–H), 12.03 (br, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 35.50 (1C, CH_2Ph), 126.29 (1C, C_{Ar}), 128.22 (2CH, C_{Ar}), 128.96 (2CH, C_{Ar}), 137.28 (1C, C_{Ar}), 140.65 (1C, C-6), 142.74 (1C, C-3), 149.57 (1C, C═O); MS (EI, 70 eV) *m/z* (%) = 229 (M^+ +1, 1.2), 228 (3.9), 171 (100.0), 153 (11.3), 131 (9.4), 117 (15.6), 103 (54.2), 91 (32.9), 77 (27.6), 53 (7.3), 52(36.2), 50 (16.6). *Anal*. Calcd for $C_{10}H_8N_6O$ (228.22): C, 52.63; H, 3.53; N, 36.83%. Found: C, 52.61; H, 3.56; N, 36.80%.

4-Amino-6-benzyl-3-((2-oxo-1,2-diphenylethylidene) hydrazono)-3,4-dihydro-1,2,4-triazin-5(2H)-one (7). To a solution of **2** (0.5 g, 2.16 mmol) and benzyl (0.45 g, 2.16 mmol) in ethanol (50 mL) containing pipridine (5 drops) was refluxed for 6 h. The product obtained on cooling was filtered off and boiled in petroleum ether then recrystallized to afford **7**.

Yellow powder (0.69 g, 75%); mp 171–172°C; [ethyl acetate]; R_f = 0.28 [pet. ether (60:80)/ethyl acetate (4:1)]; IR (KBr): ν/cm^{-1} = 3309, 3228 (NH_2), 3106 (NH), 2915 (CH_2), 1691 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.88 (s, 2H, CH_2–Ph), 5.39 (s, 2H, NH_2), 7.21–7.86 (m, 15H, Ar–H), 12.65 (br, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 35.50 (1C, CH_2–Ph), 125.50 (1CH, C_{Ar}), 127.31 (2CH, C_{Ar}), 127.53 (2CH, C_{Ar}), 128.10 (2CH, C_{Ar}), 128.36 (2CH, C_{Ar}), 129.15 (2CH, C_{Ar}), 129.55 (2CH, C_{Ar}), 130.06 (1C, C_{Ar}), 133.26 (1C, C_{Ar}), 135.04 (1C, C_{Ar}), 136.87 (1C, C_{Ar}), 142.54 (1C, C_{Ar}), 146.97 (1C, C-6), 149.74 (2C, C-3, N═C–Ph), 157.67 (1C, C═O), 198.48 (O═C–Ph); MS (EI, 70 eV) *m/z* (%) = 425 (M^+ +1, 4.1), 424 (12.6), 423 (2.0), 320 (29.7), 319 (90.7), 179 (3.2), 178 (14.9), 165 (13.9), 164 (2.1), 117 (15.4), 116(14.0), 105 (61.2), 104 (35.6), 91 (100.0), 77 (80.9),76 (11.3), 52 (2.8), 51 (21.5), 50 (3.8). *Anal*. Calcd for $C_{24}H_{20}N_6O_2$ (424.46): C, 67.91; H, 4.75; N, 19.80%. Found: C, 67.94; H, 4.73; N, 19.83%.

4-Amino-6-benzyl-3-((2-hydroxy-3H-indol-3-ylidene) hydrazono)-3,4-dihydro-1,2,4-triazin-5(2H)-one (8). Method a: A mixture of hydrazinyl **2** (0.5 g, 2.16 mmol) and isatine (0.32 g, 2.16 mmol) in ethanol (15 mL) and pipridine (4 drops) was refluxed for 6 h. The precipitate which formed on hot was separated by filtration and recrystallized to afford **8**.

Method b: A mixture of hydrazide **2** (0.5 g, 2.16 mmol) and isatine (0.32 g, 2.1 mmol) in DMF (15 mL) was refluxed for 24 h; after cooling, the reaction mixture was poured onto ice. The solid obtained was filtered, washed with hot ethanol (10 mL, 3-fold), and recrystallized to afford **8**.

Yellow flakes (0.65 g, 84%); mp 285–287°C; [DMF]; R_f = 0.26 [pet. ether (60:80)/ethyl acetate (4:2)]; IR (KBr): ν/cm^{-1} = 3410 (OH), 3310, 3200 (NH_2), 3172 (NH), 2915 (CH_2), 1679 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.01 (s, 2H, CH_2–Ph), 6.09 (s, 2H, NH_2, D_2O exchangeable), 6.94–7.41 (m, 9H, Ar–H), 10.68 (s, 1H, NH, D_2O exchangeable), 11.17 (s, 1H, OH, D_2O exchangeable); MS (EI, 70 eV) *m/z* (%) = 362 (M^+ +1, 2.1), 346 (1.0), 216 (2.1), 144 (30.9), 130 (29.0), 117 (100.0), 103 (43.6), 90 (86.0), 76 (55.6), 53 (5.0), 50 (39.4). *Anal*. Calcd for $C_{18}H_{15}N_7O_2$ (361.37): C, 59.83; H, 4.18; N, 27.13%. Found: C, 59.85; H, 4.21; N, 27.10%.

N-Acetyl-N-(2-acetyl-3-benzoyl-6-benzyl-7-oxo-3-phenyl-2,3-dihydro-1,2,4-triazolo[4,3-b] 1,2,4-triazin-8(7H)-yl)acetamide (9). A solution of **7** (0.5 g, 1.18 mmol) in 7 mL acetic anhydride was heated under reflux for 12 h. The reaction mixture was then cooled and poured into crushed ice. The precipitate formed was filtered off, washed with water (10 mL), and then recrystallized to afford **9**.

Gray powder (0.45 g, 70%); mp 180–182°C; [ethyl acetate]; R_f = 0.23 [pet. ether (60:80)/ethyl acetate (4:1)]; IR (KBr): ν/cm^{-1} = 2915 (CH_2), 1746 (O═C–Ph), 1652 (O═C–CH_3), 1644 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.93 (s, 3H, $CH_{3triazole}$), 2.50 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.85 (s, 2H, CH_2–Ph), 6.92–7.94 (m, 15H, Ar–H); ^{13}C NMR (DMSO-d_6) δ: 20.47 (1C, O═C–$\underline{C}H_3$), 23.58 (1C, O═C–$\underline{C}H_3$), 24.32 (1C, O═C–$\underline{C}H_3$), 35.60 (1C, CH_2–Ph), 87.16 (1C, $C_{3'}$), 126.57 (1$\underline{C}$H, C_{Ar}), 127.56 (2CH, C_{Ar}), 127.81 (1CH, C_{Ar}), 128.17 (4CH, C_{Ar}), 128.81 (2CH, C_{Ar}), 129.16 (2CH, C_{Ar}), 129.40 (2CH, C_{Ar}), 133.29 (1C, C_{Ar}), 133.94 (1C, C_{Ar}), 134.43 (1C, C_{Ar}), 135.31 (1C, C_{Ar}), 144.57 (1C, C_6), 151.22 (1C, C_3), 166.89 (1C, C═O), 168.10 (2C, O═$\underline{C}$–CH_3), 169.42 (1C, O═$\underline{C}$–CH_3), 188.54 (O═C–Ph); MS (EI, 70 eV) *m/z* (%) = 551 (M^+ +1, 1.0), 509 (10.8), 467 (5.5), 445 (13.9), 403 (100.0), 362 (20.6), 361 (94.3), 320 (17.5), 319 (84.4), 304 (6.9), 241 (1.4), 216 (1.5), 200 (3.4), 186 (4.5), 165 (10.5), 119 (4.7), 118 (6.2), 117 (5.5), 105 (77.5), 104 (22.4), 103 (10.1), 91 (57.9), 77 (52.9), 51 (5.1), 50 (1.2), 42 (53.5). *Anal*. Calcd for $C_{30}H_{26}N_6O_5$ (550.58): C, 65.45; H, 4.76; N, 15.26%. Found: C, 65.42; H, 4.80; N, 15.24%.

3-Benzyl-1,7-dihydro-4*H*-1,2,4-triazino[3′,4′:3,4]1,2,4,5-tetrazepino [6,7-b]indol-4-one (11). A mixture of **8** (0.6 g, 1.66 mmol) and glacial acetic acid (15 mL) and anhydrous fused sodium acetate (0.2 g) was refluxed for 24 h. After cooling, the reaction mixture was poured onto crushed ice. The obtained solid was filtered off and recrystallized to afford **11**.

Black powder (0.37 g, 65%); mp > 300°C (DMF/ethanol); R_f = 0.51 [pet. ether (60–80)/ethyl acetate (4:2)]; IR (KBr): ν/cm^{-1} = 3228 (NH), 2915 (CH_2), 1679 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.79 (s, 2H, CH_2–Ph), 4.193 (br, 1H, NH), 6.96–7.36 (m, 9H, Ar–H), 11.13 (br, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 341(M^+ −2, 30.8), 170 (69.2), 168 (38.5), 141 (46.2), 140 (61.5), 115 (76.9), 105 (61.5), 104 (61.5), 77 (46.2), 76 (53.8), 60 (100.0), 51 (92.3), 50 (53.8). *Anal*. Calcd for $C_{18}H_{13}N_7O$ (343.35): C, 62.97; H, 3.82; N, 28.56%. Found: C, 62.95; H, 3.84; N, 28.58%.

9-Amino-7-benzyl-2,9-dihydro-8*H*-1,2,4-triazino[4,3-b]1,2,4-triazine-3,8(4*H*)-dione (12). A mixture of hydrazinyl **2** (0.5 g, 2.16 mmol) and ethyl bromoacetate (0.2 mL, 1.85 mmol) in acetonitrile (15 mL) containing five drops of triethylamine was refluxed for 6 h. The precipitate which formed on hot was separated by filtration and recrystallized to afford **12**.

Yellow flakes (0.48 g, 82%); mp > 300°C; [DMF]; R_f = 0.48 [ethyl acetate/ethanol (4:2.5)]; IR (KBr): ν/cm^{-1} = 3330, 3305 (NH_2), 3244 (NH), 2915 (CH_2), 1689 (C═O amide), and 1660 (C═C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 4H, CH_2–Ph + endo cyclic CH_2), 6.03 (s, 2H, NH_2, D_2O exchangeable), 7.19–7.26 (m, 5H, Ar–H), 11.94 (s, 1H, NH, D_2O exchangeable); MS (EI, 70 eV) *m/z* (%) = 273 (M^+ +1, 7.3), 245 (14.2), 231 (11.3), 217(12.8), 201 (10.3), 186 (32.0), 115 (75.3), 91 (81.9), 76 (92.3), 66 (8), 64 (100.0), 53 (25.3), 51 (88.3). *Anal*. Calcd for $C_{12}H_{12}N_6O_2$ (272.10): C, 52.94; H, 4.44; N, 30.87%. Found: C, 52.92; H, 4.41; N; 30.85%.

Antimicrobial assay. Four bacteria, *S. pneumonia* (RCMB 010010) and *B. subtilis* (RCMB 010067) (Gram-positive bacteria) and *P. aeruginosa* (RCMB 010043) and *E. coli* (RCMB 010052) (Gram-negative bacteria) and two fungi *A. fumigates* (RCMB 02568) and *C. albicans* (RCMB 05036) produced from Regional Center for Mycology and Biotechnology Culture, were used for this study.

Antimicrobial activity (in vitro). The susceptibility tests have been performed according to the NCCLS recommendations (National Committee for Clinical Laboratory Standards, 1993). Screening tests regarding the inhibition zone have been carried out by the well diffusion method [23,24]. The inoculum suspension has been prepared from colonies grown overnight on an agar plate and inoculated into Mueller–Hinton broth (Merck, Darmstadt, Germany). A sterile swab has been immersed in the bacterial and fungal suspension and used to inoculate Mueller–Hinton agar plates.

The compounds have been dissolved in dimethyl sulfoxide (DMSO). The inhibition zone has been measured around each well after 24 h at 37°C. Controls using DMSO have been adequately done. To assess the MIC defined as the drug concentration at which no growth has been visible, 96-well sterile micro plates (Corning Inc., Corning, NY, USA) have been filled with 0.1 mL of serial twofold dilutions (500 to 0.12 μg/mL) of the naphthoquinones. The compounds have been diluted in Mueller–Hinton broth from a 10 mg/mL stock solution in DMSO. A standardized number of bacteria and fungi (0.1 mL of a 10^6 CFU/mL suspension in Mueller–Hinton broth) have been included into each well. A growth well (both plus inoculum) and a sterility control well (broth only) were included in each panel. Micro plates have been incubated at 37°C for 24 h, and then MIC was determined as the last dilution at which no increase in turbidity has been observed.

Cytotoxic evaluation (*in vitro*).

Cell line. Mammary gland breast cancer (MCF-7), hepatocellular carcinoma (HePG2), and human colon carcinoma (HCT116) have been obtained from the VACSERA Tissue Culture Unit, Cairo, Egypt.

Doxorubicin has been used as a standard anticancer drug for comparison.

Chemical reagents. Dimethyl sulfoxide, crystal violet, and trypan dye have been purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum, Dulbecco's modified Eagle's medium, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycine, and 0.25% Trypsin–EDTA have been purchased from Lonza.

Crystal violet stain (1%). It is composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with bidistilled (dd)H_2O and filtered through a Whatman no. 1 filter paper.

Cell line propagation. The cells have been propagated in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum 1% L-glutamine, HEPES buffer, and 50 mg/mL gentamicin. All cells have been maintained at 37°C in a humidified atmosphere of 5% CO_2 and have been subcultured two times a week.

Cell toxicity has been monitored by determining the effect of the test samples on cell morphology and cell viability.

Cytotoxicity evaluation using viability assay. For cytotoxicity assay, the cells have been seeded in 96-well plate at a cell concentration (10^4 cells/well in 100 mL of medium). Fresh medium containing different concentrations of the test sample has been added after 24 h of seeding. Serial twofold dilutions of the tested chemical compound have been added to confluent cell

monolayers dispensed in 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates have been incubated at 37°C in a humidified incubator with 5% CO_2 for 48 h. Three wells have been used for each concentration of the test sample. Control cells have been incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 1%) has been found not to affect to experimental conditions. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125, and 1.56 mg) have been added, and the incubation has been continued for 48 h, and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, the media has been aspirated, and the crystal violet solution (1%) has been added to each well for at least 30 min. The stain has been removed, and the plates have been rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) has then been added to all wells and mixed thoroughly, and then the absorbance of the plates has been measured after gently shaken on the microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results have been corrected for background absorbance detected in wells without added stain. Treated samples have been compared with the cell control in the absence of the tested compounds. All experiments have been carried out in triplicate. The cellular cytotoxic effect of each tested compound has been calculated [25,26].

Molecular modeling.

Geometry optimization. Geometry optimization of the molecular structures for the different compounds was carried out using the density functional theory via cluster calculations using DMOL3 program [21] in Materials Studio package [22] in order to investigate their stability relative to each other. The values resulted for the main quantum parameters are summarized in Table 8.

The orientation of 4-amino-6-benzyl-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one (**2**) exists in two tautomeric structures **2a** and **2b**. The results of binding energies calculations showed that **2b** isomer has a binding energy value with 0.18418 eV more negative than that of isomer **2a**. So the isomer **2b** is more stable than the isomer **2a**. Furthermore, the difference between the frontier orbitals ($E_{HOMO} - E_{LUMO}$) indicates that the **2b** is more chemically stable than **2a**.

Structure activity relationship. All compounds **1**–**12** revealed antimicrobial activity; however, compound **4c** was found to be more effective against only Gram negative, whereas compound **8** was found to be effective against both Gram-positive and Gram-negative bacteria. In case of antifungal activity, it was observed that compound **12** showed good activity. The MIC (μg/mL) values for most active compounds **4c** and **8** were recorded. The MIC (μg/mL) values for most active compounds **4c** and **8** were recorded. Also, study the anticarcinogenic activity of the most potent compounds **4c** and **8**. The activity of compound **4c** was increased around seven times more than that of ampicillin while compound **8** was increased around two times more than that of ampicillin due to the presence of hydroxyl this activity may be attributed to the presence of bidentate Schiff bases of β-naphthole or hydroxyindole, respectively.

CONFLICTS OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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