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Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents

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Abstract—A new series of 2-arylquinoline-4-carboxylic acid hydrazide–hydrazones was synthesized using an appropriate synthetic route. All the target compounds were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus* as an example for Gram-positive bacteria, *Escherichia coli* as an example for Gram-negative bacteria, and *Candida albicans* as a representative of fungi. The minimum inhibitory concentration (MIC) was determined for test compounds as well as for reference standards. Among the compounds tested, compounds having nitro substituents at the arylidene moiety showed the most potent antifungal as well as antibacterial activities against *E. coli*. Compound **23** displayed an antifungal activity comparable to that of nystatin. However, none of the compounds demonstrated any antibacterial activity against *S. aureus*. Hydrophobicity of the target compounds correlated weakly with their antibacterial and antifungal activities. The most potent compounds namely, **7**, **18**, **19**, **22**, and **23** were assessed for hemolytic toxicity and found to be non-hemolytic up to a concentration of 100 μg/mL. In addition, the most potent compound (**23**) was evaluated for in vitro cytotoxic activity against various cancer cell lines. This compound was found to display no cytotoxic activity but rather it induces the proliferation rate of Hep-G2 cells.

1. Introduction

The dramatically rising prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. In particular, the emergence of multi-drug resistant strains of Gram-positive bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is a problem of ever-increasing significance.[1–5] One way to counterbalance this challenge is the controlled use of the currently marketed antibiotics; the other is the development of novel antimicrobial agents. Consequently, the search for new antimicrobial agents will always remain an important and challenging task for medicinal chemists.

Being a structural building block in many natural products, the quinoline ring is one of the most commonly encountered heterocycles in medicinal chemistry. Literature survey revealed that substituted quinolines possess diverse chemotherapeutic activities including antibacterial,[6,7] antifungal,[8,9] antiamoebic,[10,11] antileishmanial,[12,13] antimalarial,[14,15] and antitumor activities.[16,17] Meanwhile, hydrazide–hydrazones have been claimed to exhibit appreciable antimicrobial activity.[18–21] On the basis of these observations, we had the impetus to synthesize a number of hydrazide–hydrazones of the synthetically accessible 2-arylquinoline-4-carboxylic acids and subsequently evaluate their in vitro antimicrobial activity.

2. Chemistry

The synthesis of the target compounds was carried out as outlined in Scheme 1. The versatile Pfitzinger reaction[22] was utilized to synthesize the starting 2-arylquinoline-4-carboxylic acids (**1a–j**) in satisfactory yields by reacting isatin or substituted isatins with

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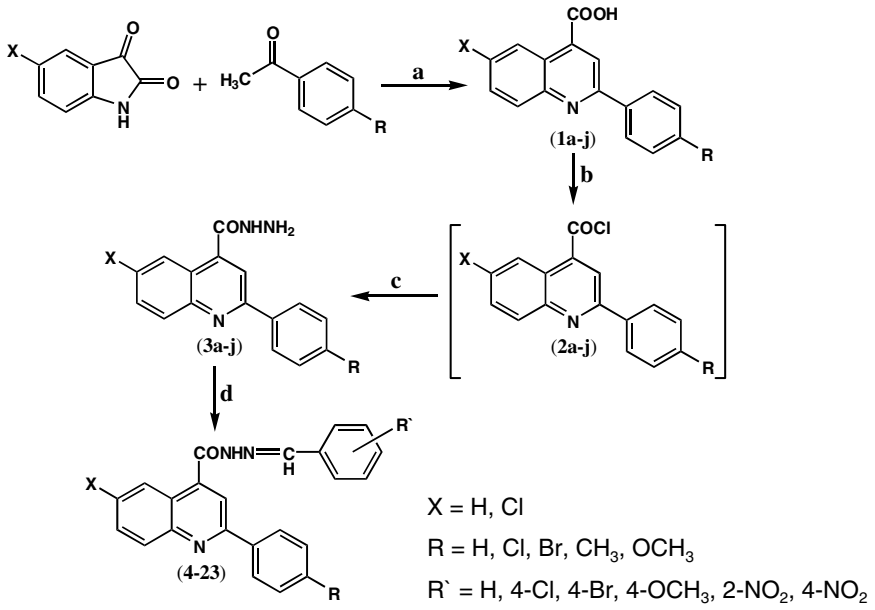

Scheme 1. Synthetic route for the target compounds. Reagents and conditions: (a) NaOH, aq EtOH; (b) $SOCl_2$, dry benzene; (c) $NH_2NH_2{\cdot}H_2O$, EtOH; (d) substituted benzaldehydes, HOAc.

α-methylketones in aqueous ethanol. The acids were subsequently treated with thionyl chloride in refluxing benzene to give the corresponding acid chlorides (**2a–j**) which were used directly to prepare the hydrazides (**3a–j**) through reaction with hydrazine hydrate in refluxing ethanol. The requisite hydrazones (**4–23**) were obtained by condensing the acid hydrazides (**3a–j**) with the appropriate aldehydes in glacial acetic acid under reflux conditions.

The new hydrazones were characterized by their physical, analytical, and spectral data. IR spectra of the target hydrazones showed NH and C=O stretching bands at 3140–3190 and 1650–1660 cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected regions. In the ^1H NMR spectra of the target hydrazones, the azomethine proton appeared at 8.3–8.6 ppm as a sharp D_2O non-exchangeable singlet, whereas a broad more downfield D_2O exchangeable singlet at 12.1–12.6 ppm was characteristic of the NH group. The other protons appeared at the expected chemical shifts and integral values. All the target hydrazones were microanalyzed satisfactorily for C, H, and N.

3. Results and discussion

All the newly synthesized hydrazones were assayed in vitro for antibacterial activity against *S. aureus* (example for Gram-positive bacteria) and *Escherichia coli* (example for Gram-negative bacteria), and the antifungal activity was evaluated against *Candida albicans*. The MIC values were determined by the twofold serial dilution technique in Mueller–Hinton broth and Sabouraud dextrose broth for the antibacterial and antifungal assay, respectively. For comparison, ampicillin was used as the reference antibacterial agent and nystatin was employed as the reference antifungal agent. The MIC values for test compounds as well as reference standards are given in Table 1.

The obtained results revealed that the nature of substituents and substitution pattern on the quinoline ring may have a considerable impact on the antibacterial and antifungal activities of the target hydrazones. Of particular importance, a nitro group in the arylidene side chain generally enhances the antifungal activity and antibacterial activity against *E. coli*. In this context, para substitution appears to be more beneficial for activity compared to the ortho substitution pattern. In the absence of nitro substituents in the arylidene moiety, however, the introduction of a chloro group at C-6 of the quinoline nucleus imparts appreciable antibacterial activity against *E. coli*. On the other hand, the nature of substituent at the 2-phenyl ring appears to have little influence on antimicrobial activity. Consistent with these results, it was not surprising that compound **23** was found to be the most potent among the test hydrazones. Interestingly, all the target compounds were found to be devoid of antibacterial activity against *S. aureus*. Log *P* values were calculated for the target compounds using ACD/LogP software and plotted against antibacterial and antifungal activities expressed as −log MIC values as illustrated in Figures 1 and 2. The hydrophobic character of the test compounds correlated weakly with antibacterial ($r = 0.38$) and antifungal ($r = 0.11$) activities with lipophilicity optima of approximately 4.98 and 5.31, respectively.

Compounds that exhibited the most potent antibacterial and antifungal activities namely, **7**, **18**, **19**, **22**, and **23**, were evaluated for hemolytic toxicity and minimal hemolytic concentrations (MHCs) were determined. Up to a concentration of 100 μg/mL, the test compounds showed no hemolytic activity. Even at as high

Table 1. MIC values for the target hydrazones

Compound	log P[a]	MIC (μg/mL)		
		Gram-positive bacteria *Staph. Aureus* ATCC 25923	Gram-negative bacteria *E. coli* ATCC 10536	Fungi *C. albicans* ATCC 10231
4	5.04 ± 0.41	>200	>200	50–100
5	5.97 ± 0.48	>200	>200	50–100
6	6.15 ± 0.54	>200	>200	100–200
7	5.32 ± 0.49	>200	25–50	25–50
8	4.29 ± 0.41	>200	>200	100–200
9	5.22 ± 0.48	>200	>200	50
10	5.69 ± 0.40	>200	>200	50–100
11	5.12 ± 0.41	>200	50	50–100
12	5.87 ± 0.42	>200	50–100	50–100
13	6.05 ± 0.49	>200	100	25–50
14	5.58 ± 0.41	>200	50	50–100
15	6.44 ± 0.43	>200	50	50–100
16	5.81 ± 0.44	>200	50–100	50–100
17	5.87 ± 0.48	>200	100	100–200
18	5.97 ± 0.51	>200	25–50	50–100
19	5.79 ± 0.50	>200	25–50	50
20	5.69 ± 0.43	>200	50	50
21	5.87 ± 0.50	>200	50	25–50
22	5.03 ± 0.44	>200	25–50	25–50
23	4.85 ± 0.44	>200	25–50	25
Ampicillin	—	12.5	25	—
Nystatin	—	—	—	25

[a] log P values were calculated using ACD/LogP software.

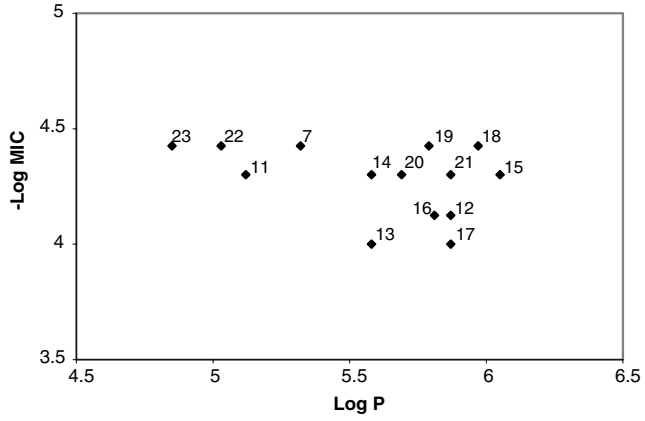

Figure 1. Plot of hydrophobicity of the test compounds versus antibacterial activity against *E. coli*.

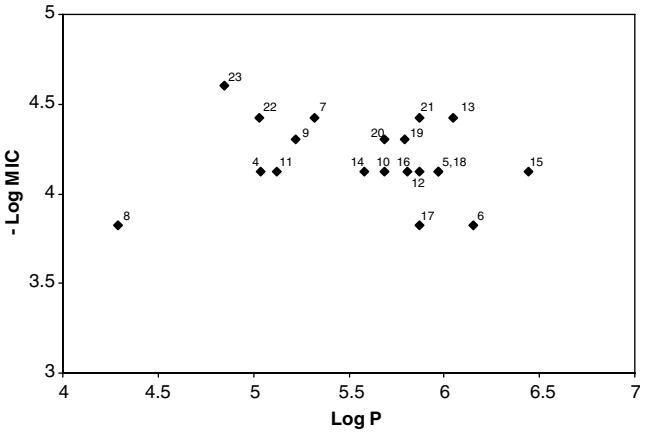

Figure 2. Plot of hydrophobicity of the test compounds versus antifungal activity.

concentration as 1 mg/mL, the tested compounds exhibited only 5% hemolysis (Fig. 3). Furthermore, compound **23**, being the most potent in the series, was evaluated for in vitro cytotoxic activity against Hep-G2, MCF-7, and 1301 cell lines. This compound led to a significant induction of the proliferation rate of Hep-G2 cells ($P < 0.01$) to around 1.6-fold of the control cells and this maximum induction seems unchanged with the increasing of the dose. The tested compound administration to Raw macrophage 264.7 resulted in a significant elevation in growth rate ($P < 0.05$), while it exhibited insignificant increment in the cell growth of lymphoblastic leukemia (1301). On the other hand, the tested doses have led to static unchangeable rate of growth in the treated MCF-7 cells (Fig. 4).

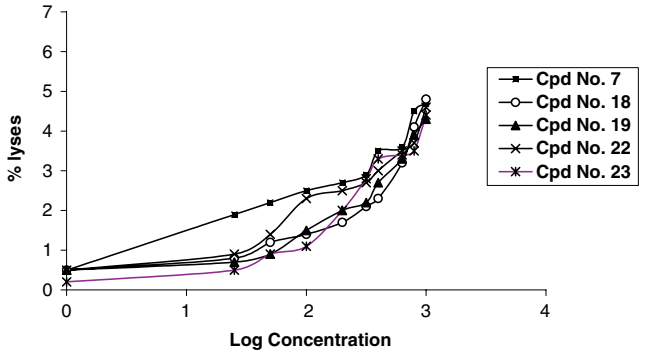

Figure 3. Plot of log concentration versus percentage lyses of test compounds **7**, **18**, **19**, **22**, and **23**.

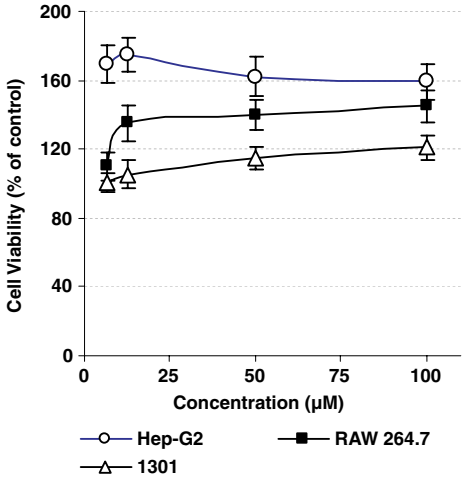

Figure 4. Plot of concentration of test compounds versus cell viability.

4. Experimental protocols

4.1. Syntheses

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Pye Unicam SP 1000 IR spectrophotometer as KBr pellets. ^{1}H NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer in DMSO-d_6. Chemical shifts were expressed in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. MS spectra were measured with an HP 5995 instrument. Elemental analyses (C, H, and N) were performed at the National Research Centre, Cairo, Egypt. All compounds were routinely checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates. All solvents were dried by standard methods. Compounds (**1a–j**)[23–27] and (**3e–g**)[23,24,28–30] were synthesized according to literature procedures.

4.2. General method for the synthesis of 6-substituted-2-arylquinoline-4-carboxylic acid hydrazides (3a–d)

A mixture of 6-substituted-2-arylquinoline-4-carboxylic acids (**1a–d**; 10 mmol), thionyl chloride (2.4 g, 20 mmol) in dry benzene (50 mL) was heated at reflux for 4 h. Benzene was distilled under reduced pressure and the residue was dissolved in ethanol (50 mL), hydrazine hydrate (85%; 3 mL) was added, and the mixture was heated at reflux for 4 h. The reaction mixture was evaporated under reduced pressure and the crude product was purified by recrystallization from ethanol.

4.3. 6-Chloro-2-(4-chlorophenyl)quinoline-4-carboxylic acid hydrazide (3a)

Yield 77%; mp 256–258 °C (EtOH); IR (KBr, cm^{-1}): 3265 (NHs), 3057, 2924, 1642 (C=O), 1586, 1540, 1488, 1090, 886, 830, 723. ^{1}H NMR (DMSO-d_6) δ: 4.9 (s, 2H, NH_2, D_2O-exchangeable), 7.5–7.7 (m, 2H, Ar–H), 7.8–7.9 (m, 1H, Ar–H), 8.1–8.2 (m, 2H, Ar–H), 8.3–8.4 (d, 2H, Ar–H), 8.8–8.9 (d, 1H, Ar–H), 9.9 (s, 1H, CONH, D_2O-exchangeable). Anal. Calcd for $C_{16}H_{11}Cl_2N_3O$: C, 57.85; H, 3.34; N, 12.65. Found: C, 58.00; H, 3.39; N, 12.54.

4.4. 6-Chloro-2-(4-bromophenyl)quinoline-4-carboxylic acid hydrazide (3b)

Yield 82%; mp 253–255 °C (EtOH); IR (KBr, cm^{-1}): 3268 (NHs), 3048, 2922, 1640 (C=O), 1586, 1542, 1485, 1074, 885, 828, 722. ^{1}H NMR (DMSO-d_6) δ: 5.3 (s, 2H, NH_2, D_2O-exchangeable), 7.4–7.6 (m, 2H, Ar–H), 7.6–7.7 (m, 1H, Ar–H), 7.8–7.9 (m, 1H, Ar–H), 8.2–8.3 (m, 2H, Ar–H), 8.3 (s, 1H, Ar–H), 9.1–9.2 (d, 1H, Ar–H), 9.8 (s, 1H, CONH, D_2O-exchangeable). Anal. Calcd for $C_{16}H_{11}BrClN_3O$: C, 51.02; H, 2.94; N, 11.16. Found: C, 51.36; H, 3.18; N, 11.24.

4.5. 6-Chloro-2-(4-methylphenyl)quinoline-4-carboxylic acid hydrazide (3c)

Yield 69%; mp 249–251 °C (EtOH); IR (KBr, cm^{-1}): 3261 (NHs), 3057, 2926, 1639 (C=O), 1586, 1542, 1489, 1445, 1352, 1036, 885, 754, 704. ^{1}H NMR (DMSO-d_6) δ: 2.4 (s, 3H, CH_3), 5.1 (s, 2H, NH_2), 7.3–7.4 (d, 2H, Ar–H), 7.8–7.9 (d, 1H, Ar–H), 8.1–8.2 (m, 3H, Ar–H), 8.4 (s, 1H, Ar–H), 8.6 (s, 1H, Ar–H), 9.9 (s, 1H, CONH). Anal. Calcd for $C_{17}H_{14}ClN_3O$: C, 65.49; H, 4.53; N, 13.48. Found: C, 65.50; H, 4.85; N, 13.73.

4.6. 6-Chloro-2-(4-methoxyphenyl)quinoline-4-carboxylic acid hydrazide (3d)

Yield 68%; mp 233–236 °C (EtOH); IR (KBr, cm^{-1}): 3282 (NHs), 3034, 2962, 1634 (C=O), 1584, 1542, 1517, 1350, 1250, 1174, 1033, 835, 732. ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, CH_3), 4.7 (s, 2H, NH_2, D_2O exchangeable), 7.1–7.2 (d, 2H, Ar–H), 7.7–7.8 (d, 1H, Ar–H), 8.0–8.1 (m, 2H, Ar–H), 8.2–8.3 (m, 3H, Ar–H), 10.1 (s, 1H, CONH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.01; H, 4.70; N, 13.13.

4.7. General method for the synthesis of 6-substituted-2-arylquinoline-4-carboxylic acid hydrazide–hydrazones (4–23)

A mixture of 6-substituted-2-arylquinoline-4-carboxylic acid hydrazides (**3a–j**; 10 mmol) and the appropriate aromatic aldehyde (10 mmol) in glacial acetic acid (50 mL) was heated at reflux for 4–8 h. The reaction mixture was concentrated under reduced pressure, cooled, and the obtained solid was filtered, washed with water and cold ethanol. The crude products were purified by recrystallization from the appropriate solvent.

4.8. 2-(4-Chlorophenyl)quinoline-4-carboxylic acid benzylidene-hydrazide (4)

Yield 65%; mp 239–241 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.2 (m, 1H, Ar–H), 7.4–7.5 (m, 2H, Ar–H), 7.6–7.9 (complex m, 6H, Ar–H), 8.1–8.3 (m, 2H, Ar–H), 8.3–8.5 (m, 4H, Ar–H), 12.2 (s, 1H, CONH). Anal. Calcd for $C_{23}H_{16}ClN_3O$: C, 71.60; H, 4.18; N, 10.89. Found: C, 71.31; H, 4.16; N, 10.72.

4.9. 2-(4-Bromophenyl)quinoline-4-carboxylic acid (4-chlorobenzylidene)-hydrazide (5)

Yield 73%; mp 269–271 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.3 (dd, 1H, Ar–H), 7.5–7.6 (d, 1H, Ar–H), 7.6–7.9 (m, 5H, Ar–H), 8.1–8.3 (m, 3H, azomethine CH, Ar–H), 8.3–8.5 (m, 4H, Ar–H), 12.3 (s, 1H, CONH). MS (*m*/*z*): 465 (M^+, 14.1%), 326 (54.7%), 312 (42.9%), 284 (53.2%), 203 (base peak), 101 (43.6%), 75 (64.0%). Anal. Calcd for $C_{23}H_{15}BrClN_3O$: C, 59.44; H, 3.25; N, 9.04. Found: C, 59.29; H, 3.07; N, 8.94.

4.10. 2-(4-Bromophenyl)quinoline-4-carboxylic acid (4-bromobenzylidene)-hydrazide (6)

Yield 80%; mp 264–266 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.2 (d, 1H, Ar–H), 7.2–7.3 (d, 1H, Ar–H), 7.5–7.7 (m, 4H, Ar–H), 7.8–8.0 (m, 3H, Ar–H), 8.1–8.3 (m, 2H, Ar–H), 8.3–8.5 (m, 3H, azomethine CH, Ar–H), 12.3 (s, 1H, CONH). Anal. Calcd for $C_{23}H_{15}Br_2N_3O$: C, 54.25; H, 2.97; N, 8.25. Found: C, 54.15; H, 3.06; N, 8.16.

4.11. 2-(4-Bromophenyl)quinoline-4-carboxylic acid (2-nitrobenzylidene)-hydrazide (7)

Yield 66%; mp 259–261 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.2 (d, 1H, Ar–H), 7.4–7.5 (m, 1H, Ar–H), 7.6–7.8 (m, 3H, Ar–H), 7.8–7.9 (m, 2H, Ar–H), 8.1–8.3 (m, 3H, Ar–H), 8.2–8.3 (m, 2H, Ar–H), 8.4 (s, 1H, azomethine CH), 8.8 (s, 1H, Ar–H), 12.5 (s, 1H, CONH). Anal. Calcd for $C_{23}H_{15}BrN_4O_3$: C, 58.12; H, 3.18; N, 11.79. Found: C, 58.00; H, 2.92; N, 11.58.

4.12. 2-(4-methoxyphenyl)quinoline-4-carboxylic acid benzylidene-hydrazide (8)

Yield 67%; mp 257–260 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.0–7.1 (d, 2H, Ar–H), 7.6–7.7 (d, 2H, Ar–H), 7.8–7.9 (d, 2H, Ar–H), 8.2–8.3 (d, 2H, Ar–H), 8.3–8.5 (m, 5H, azomethine CH, Ar–H), 12.1 (s, 1H, CONH). Anal. Calcd for $C_{24}H_{19}N_3O_2$: C, 75.57; H, 5.02; N, 11.02. Found: C, 75.23; H, 5.37; N, 10.86.

4.13. 2-(4-Methoxyphenyl)quinoline-4-carboxylic acid (4-bromobenzylidene)-hydrazide (9)

Yield 75%; mp 278–280 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.8 (s, 3H, CH_3), 7.1–7.2 (m, 3H, Ar–H), 7.4–7.5 (d, 1H, Ar–H), 7.5–7.8 (complex m, 5H, Ar–H), 8.1–8.2 (m, 2H, Ar–H), 8.3–8.4 (m, 3H, Ar–H), 12.2 (s, 1H, CONH). Anal. Calcd for $C_{24}H_{18}BrN_3O_2$: C, 62.62; H, 3.94; N, 9.13. Found: C, 62.27; H, 3.72; N, 8.78.

4.14. 2-(4-Methylphenyl)quinoline-4-carboxylic acid (4-chlorobenzylidene)-hydrazide (10)

Yield 74%; mp 241–243 °C (EtOH/H_2O); ^{1}H NMR (DMSO-d_6) δ: 1.8 (s, 3H, CH_3), 7.1–7.3 (d, 2H, Ar–H), 7.3–7.6 (m, 4H, Ar–H), 7.7–7.8 (d, 1H, Ar–H), 7.8–7.9 (d, 1H, Ar–H), 7.9–8.3 (complex m, 5H, Ar–H), 8.4 (s, 1H, azomethine CH), 12.3 (s, 1H, CONH). Anal. Calcd for $C_{24}H_{18}ClN_3O$: C, 72.09; H, 4.54; N, 10.51. Found: C, 71.84; H, 4.35; N, 10.32.

4.15. 6-Chloro-2-phenylquinoline-4-carboxylic acid benzylidene-hydrazide (11)

Yield 68%; mp 244–246 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.3 (m, 2H, Ar–H), 7.4–7.6 (m, 3H, Ar–H), 7.6–7.8 (m, 5H, Ar–H), 8.1-8.4 (m, 5H, azomethine CH, Ar–H), 12.2 (s, 1H, NH, D_2O-exchangeable). Anal. Calcd for $C_{23}H_{16}ClN_3O$: C, 71.60; H, 4.18; N, 10.89. Found: C, 71.64; H, 4.19; N, 10.82.

4.16. 6-Chloro-2-phenylquinoline-4-carboxylic acid (4-chlorobenzylidene)-hydrazide (12)

Yield 71%; mp 276–277 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.3 (dd, 1H, Ar–H), 7.5–7.7 (m, 4H, Ar–H), 7.7–7.9 (m, 3H, Ar–H), 8.1–8.3 (m, 3H, azomethine CH, Ar–H), 8.3–8.5 (m, 3H, Ar–H), 12.3 (s, 1H, NH). Anal. Calcd for $C_{23}H_{15}Cl_2N_3O$: C, 65.73; H, 3.60; N, 10.00. Found: C, 65.65; H, 3.69; N, 9.99.

4.17. 6-Chloro-2-phenylquinoline-4-carboxylic acid (4-bromobenzylidene)-hydrazide (13)

Yield 63%; mp 262–264 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.3 (dd, 1H, Ar–H), 7.5–7.7 (m, 5H, Ar–H), 7.8–8.0 (m, 3H, Ar–H), 8.1–8.3 (m, 2H, Ar–H), 8.3–8.5 (m, 3H, Ar–H), 12.3 (s, 1H, NH). Anal. Calcd for $C_{23}H_{15}BrClN_3O$: C, 59.44; H, 3.25; N, 9.04. Found: C, 59.73; H, 3.04; N, 8.92.

4.18. 6-Chloro-2-(4-methylphenyl)quinoline-4-carboxylic acid benzylidene-hydrazide (14)

Yield 70%; mp 291–294 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 7.2–7.3 (m, 2H, Ar–H), 7.4–7.5 (m, 2H, Ar–H), 7.6–7.9 (m, 5H, Ar–H), 8.1–8.3 (m, 2H, Ar–H), 8.3-8.4 (m, 2H, Ar–H), 8.4 (s, 1H, azomethine CH), 12.2 (s, 1H, NH). Anal. Calcd for $C_{24}H_{18}ClN_3O$: C, 72.09; H, 4.54; N, 10.51. Found: C, 71.76; H, 4.16; N, 10.91.

4.19. 6-Chloro-2-(4-chlorophenyl)quinoline-4-carboxylic acid (4-chlorobenzylidene)-hydrazide (15)

Yield 79%; mp 287–289 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.2–7.3 (dd, 1H, Ar–H), 7.4–7.5 (m, 2H, Ar–H), 7.6–7.7 (m, 2H, Ar–H), 7.8–7.9 (m, 2H, Ar–H), 8.1–8.2 (m, 1H, Ar–H), 8.3 (s, 1H, azomethine CH), 8.3–8.4 (m, 3H, Ar–H), 8.5 (s, 1H, Ar–H), 12.3 (s, 1H, NH). Anal. Calcd for $C_{23}H_{14}Cl_3N_3O$: C, 60.75; H, 3.10; N, 9.24. Found: C, 60.37; H, 3.04; N, 8.92.

4.20. 6-Chloro-2-(4-chlorophenyl)quinoline-4-carboxylic acid (4-methoxybenzylidene)-hydrazide (16)

Yield 72%; mp 274–276 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.1–7.2 (m, 2H, Ar–H), 7.5–7.6 (m, 1H, Ar–H), 7.8–7.9 (m, 2H, Ar–H), 8.1–8.2 (m, 3H, Ar–H), 8.2–8.4 (m, 4H, Ar–H), 8.4 (s, 1H, azomethine CH), 12.4 (s, 1H, NH). Anal.

Calcd for $C_{24}H_{17}Cl_2N_3O_2$: C, 64.01; H, 3.81; N, 9.33. Found: C, 63.97; H, 4.07; N, 9.38.

4.21. 6-Chloro-2-(4-bromophenyl)quinoline-4-carboxylic acid benzylidene-hydrazide (17)

Yield 69%; mp 277–279 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.1–7.3 (dd, 1H, Ar–H), 7.6–7.8 (m, 5H, Ar–H), 7.8–8.0 (m, 2H, Ar–H), 8.1–8.3 (m, 2H, Ar–H), 8.3–8.5 (m, 4H, Ar–H), 12.3 (s, 1H, NH). Anal. Calcd for $C_{23}H_{15}BrClN_3O$: C, 59.44; H, 3.25; N, 9.04. Found: C, 59.75; H, 3.60; N, 8.66.

4.22. 6-Chloro-2-(4-bromophenyl)quinoline-4-carboxylic acid (2-nitrobenzylidene)-hydrazide (18)

Yield 62%; mp 284–286 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.2–7.3 (d, 1H, Ar–H), 7.5–7.6 (m, 1H, Ar–H), 7.7–8.0 (m, 4H, Ar–H), 8.1–8.3 (m, 3H, Ar–H), 8.3–8.4 (m, 2H, Ar–H), 8.5 (s, 1H, azomethine CH), 8.8 (s, 1H, Ar–H), 12.6 (s, 1H, NH). Anal. Calcd for $C_{23}H_{14}ClBrClN_4O_3$: C, 54.19; H, 2.77; N, 10.99. Found: C, 54.00; H, 2.64; N, 10.71.

4.23. 6-Chloro-2-(4-bromophenyl)quinoline-4-carboxylic acid (4-nitrobenzylidene)-hydrazide (19)

Yield 74%; mp 298–300 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 7.4–7.5 (m, 1H, Ar–H), 7.6–7.8 (m, 3H, Ar–H), 7.8–7.9 (m, 2H, Ar–H), 8.1–8.3 (m, 3H, Ar–H), 8.3–8.4 (m, 2H, Ar–H), 8.5 (s, 1H, azomethine CH), 8.8 (s, 1H, Ar–H), 12.5 (s, 1H, NH). Anal. Calcd for $C_{23}H_{14}BrClN_4O_3$: C, 54.19; H, 2.77; N, 10.99. Found: C, 53.65; H, 2.77; N, 10.99.

4.24. 6-Chloro-2-(4-methoxyphenyl)quinoline-4-carboxylic acid (4-chlorobenzylidene)-hydrazide (20)

Yield 72%; mp 295–297 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.01–7.2 (d, 2H, Ar–H), 7.4–7.5 (complex m, 5H, Ar–H), 7.5–7.9 (m, 2H, Ar–H), 8.1–8.2 (m, 2H, Ar–H), 8.3–8.4 (m, 3H, azomethine CH, Ar–H), 12.1 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}Cl_2N_3O_2$: C, 64.01; H, 3.81; N, 9.33. Found: C, 63.62; H, 3.73; N, 9.48.

4.25. 6-Chloro-2-(4-methoxyphenyl)quinoline-4-carboxylic acid (4-bromobenzylidene)-hydrazide (21)

Yield 69%; mp >300 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.0–7.2 (m, 3H, Ar–H), 7.4–7.5 (d, 1H, Ar–H), 7.6–7.8 (m, 3H, Ar–H), 7.8–7.9 (m, 1H, Ar–H), 8.1–8.2 (d, 1H, Ar–H), 8.2 (s, 1H, azomethine CH), 8.3–8.4 (m, 3H, Ar–H), 12.3 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}BrClN_3O_2$: C, 58.26; H, 3.46; N, 8.49. Found: C, 57.89; H, 3.37; N, 8.65.

4.26. 6-Chloro-2-(4-methoxyphenyl)quinoline-4-carboxylic acid (2-nitrobenzylidene)-hydrazide (22)

Yield 62%; mp 258–260 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.1–7.2 (d, 2H, Ar–H), 7.7–7.8 (m, 1H, Ar–H), 7.8–8.0 (m, 2H, Ar–H), 8.1–8.2 (m, 2H, Ar–H), 8.2–8.3 (m, 2H, Ar–H), 8.3–8.4 (m, 2H, Ar–H), 8.4 (s, 1H, azomethine CH), 8.8 (s, 1H, Ar–H), 12.6 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}ClN_4O_4$: C, 62.55; H, 3.72; N, 12.16. Found: C, 62.19; H, 3.47; N, 11.99.

4.27. 6-Chloro-2-(4-methoxyphenyl)quinoline-4-carboxylic acid (4-nitrobenzylidene)-hydrazide (23)

Yield 71%; mp 287–290 °C (DMF/EtOH); ^{1}H NMR (DMSO-d_6) δ: 3.9 (s, 3H, OCH_3), 7.0–7.2 (m, 3H, Ar–H), 7.4–7.5 (d, 2H, Ar–H), 7.8–7.9 (m, 2H, Ar–H), 8.4 (s, 1H, azomethine CH), 8.1–8.6 (complex m, 5H, Ar–H), 12.6 (s, 1H, NH). Anal. Calcd for $C_{24}H_{17}ClN_4O_4$: C, 62.55; H, 3.72; N, 12.16. Found: C, 62.56; H, 4.04; N, 12.14.

4.28. Biology

4.28.1. Antimicrobial activity. All the test compounds were assayed in vitro for antibacterial activity against *S. aureus* ATCC 25923 (representative for Gram-positive bacteria) and *E. coli* ATCC 10536 (representative for Gram-negative bacteria), and the antifungal activity was evaluated against *C. albicans* ATCC 10231. The strains used in this study were maintained at the Microbiology Department, Faculty of Pharmacy, Zagazig University. The MIC was determined by using twofold serial dilution method.[31–33] Ampicillin and Nystatin were used as reference standards to compare the antibacterial and antifungal activities, respectively. For determining both antibacterial and antifungal activities, the synthesized compounds and the control drugs were dissolved in a mixture of sterile water and dimethylformamide (8:2, the stock solution 5 mg/mL). Further dilutions were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL concentrations. In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing broth supplemented with only DMF at the same dilution used in our experiment. The MIC values were obtained from the lowest concentration of the test compound where the tubes remain clear, indicating that the bacterial growth was completely inhibited at this concentration. The MIC values were expressed in μg/mL and the results are shown in Table 1.

4.28.1.1. Antibacterial assay. The cultures were obtained in Mueller–Hinton broth for all the bacteria after 24 h of incubation at 37 °C. Testing was carried out on Mueller–Hinton broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^6 CFU/mL. A set of tubes containing only inoculated broth was kept as control. After incubation for 24 h at 37 °C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/mL. Every experiment in the antibacterial assay was replicated twice in order to define the MIC values.

4.28.1.2. Antifungal assay. The yeast *C. albicans* was maintained in Sabouraud dextrose broth after incubation for 24 h at 35 °C. Testing was performed in Sabouraud dextrose broth at pH 7.4 and the twofold serial

dilution technique was applied. The final inoculum size was 10^5 CFU/mL. A set of tubes containing only inoculated broth was kept as control. After incubation for 48 h at 35 °C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/mL. Every experiment in the antifungal assay was replicated twice in order to define the MIC values.

4.28.2. Hemolytic assay. Fresh human blood (5 mL) was centrifuged at 700*g* (3500 rpm) for 10 min and the erythrocytes (RBCs) were collected. The erythrocytes were washed and centrifuged three times with phosphate-buffered saline (PBS, pH 7.4, 50 mL) and the supernatant was carefully decanted. The erythrocyte suspension was diluted twice with saline solution and total erythrocytes were counted using hemocytometer. Finally necessary dilution was carried out with saline solution to get total count to 6.4×10^8 cells/mL. The stock solution of the test compounds was made at an initial concentration of 4 mg/mL using the same saline solution. Assay was carried out in 1 mL Eppendorf tubes. In an Eppendorf, varying volumes (900–650 μL) of the saline solution were added and subsequently varying volumes of the stock solution of test compounds (1–250 μL) were added followed by 100 μL RBC suspension. Plain saline solution was used as a negative control, whereas Triton X-100 (10 μL of 20% w/v) was used as a positive control that produces total cell lyses. The Eppendorf tubes were then incubated at 37 °C for 30 min, centrifuged at 4000 rpm for 10 min, and the supernatant was carefully transferred to fresh Eppendorf tubes. The absorbance of the samples was read at 404 nm with LKB-Biochrom Novaspec-1049 spectrophotometer. The percentage lyses was determined according to the following equation:

$$\% \text{ Lysis} = (A_{\text{test compounds}} - A_{\text{blank}})/(A_{\text{positive control}} - A_{\text{blank}}) \times 100.$$

Percentage lyses was plotted against Log concentration of test compounds used (Fig. 3) and from the graph MHC and LD50 (concentration required for 50% lyses) was determined.[34]

4.28.3. In vitro cytotoxicity assay

4.28.3.1. Cell culture. Several human cell lines were used in testing anticancer activity including: hepatocarcinoma (Hep G2) and breast carcinoma (MCF-7), both were generous gifts from Prof. Mohamad A. Ali, Virology laboratory, NRC, Cairo, Egypt, and lymphoblastic leukemia (1301), a generous gift from The Training Center of DakoCytomation, Elly, UK, in addition to Raw murine macrophage (RAW 264.7), ATCC, VA, USA. Cells were routinely cultured in DMEM (Dulbecco's modified Eagle's medium), except RAW 264.7 cells, which were grown in RPMI-1640 at 37 °C in humidified air containing 5% CO_2. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 U/mL penicillin G sodium, 100 U/mL streptomycin sulfate, and 250 ng/mL amphotericin B. Monolayer cells were harvested by trypsin/EDTA treatment, except RAW 264.7 cells, which were collected by gentle scraping. The compounds were dissolved in dimethylsulfoxide (DMSO, 99.9%, HPLC grade) and diluted 1000-fold in the assay. The experiment was repeated four times, and the data were represented as (means ± SD). Results were compared with those of DMSO-treated cells. All culture material was obtained from Cambrex BioScience (Copenhagen, Denmark), and all chemicals were from Sigma (USA).

4.28.3.2. MTT assay. Antiproliferative activity against various tumor cell lines was estimated by the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells.[35] Cells (5×10^4 cells/well) of different cell lines were incubated with various concentrations of the tested compound at 37 °C in a serum-free medium, before submitted to MTT assay. The relative cell viability was expressed as the mean percentage of viable cells compared with DMSO-treated cells (Fig. 4).

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