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Click synthesis of new 7-chloroquinoline derivatives by using ultrasound irradiation and evaluation of their biological activity

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ABSTRACT

This study describes the click synthesis of new 7-chloroquinoline derivatives by using ultrasound irradiation and evaluation of their activity as antimicrobial, antimalarial and the anticancer. All the compounds show moderate antimalarial activity with $IC_{50} < 100$ μM, six of them showed high antimalarial activity (**2**, **3**, **4**, **6**, **8** and **9**) with $IC_{50} < 50$ μM. The most active 7-chloroquinoline derivative is a compound (**9**). Also, the newly synthesized compounds were screened for their antitumor activity towards three lines of cancer cells, **MCF-7** (human breast cancer), **HCT-116** (colon carcinoma) and **Hela** (Cervical carcinoma) cell lines. Compounds (**3**) and (**9**) exerted the highest activity on all cell lines and showed special selectivity toward **MCF-7** cells and the antibacterial screening data showed moderate to good inhibition zone (12.5 ± 0.63–23.8 ± 1.5) towards all the tested compounds. Elucidation of the structures of these new pure compounds was based on, IR, ^{1}H NMR, ^{13}C NMR, MS and their elemental analysis.
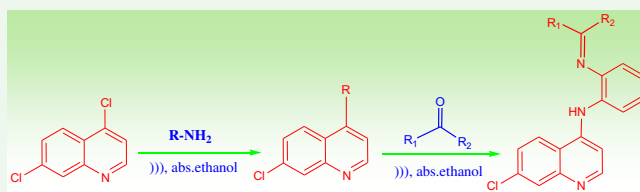
1. Introduction

Quinolines and their derivatives are present in numerous natural products and have highly antimalaria, antiasthmatic, antiinflammatory, antibacterial and antihypersitive activities (*1*). Few methods have been reported for the preparation of quinolines derivatives such as the Skraup, Doebner von Miller and Combes procedures (*2*, *3*). Malaria is a contagious disease, caused by protozoa parasites from the genus *Plasmodium* that is transmitted by mosquitoes of the genus *Anopheles*. *Plasmodium falciparum* is responsible for the most lethal form of malaria (*4*). Chloroquine was the most effective antimalarial clinically used drug but parasite resistance led to its substitution by artemisinin and its semi-synthetic derivatives (artemether, artesunate) (*5*, *6*). So, new drugs to treat malaria are critically required. Synthesis of molecular hybrids containing different moieties which are representatives of known or putative antimalarial compounds is presently being extensively explored. Recently, the synthesis of 1,2,3-triazoles by a process known as Cu-mediated click chemistry (*7*) has been explored to combine different molecules affording new analogs of chloroquine (*8*), chalcones (*9*), naphthoquinones (*10*) several other hybrid antimalarial molecules have been synthesized (*11–13*).

In this sense, ultrasonic irradiation has picked up ubiquity in the previous decades as a useful tool in most of the industrial and academic applications. The utilization of sanction as the energy source of organic synthesis (sonochemistry) is very much recorded (*14*). This energy source has been proven to be able to accelerate reactions and selectivities through the development of new receptive intermediates and compounds not usually observed under ordinary thermal conditions (*14*). Furthermore, ultrasound irradiation can be considered as earth amiable procedures, being less vitality concentrated and creating decreased amounts of side items (*15*).

In this study, the sonochemical technique was used to synthesize new derivatives of 7-chloroquinoline and evaluation as antimicrobial, antimalarial and the anticancer activity of the new compounds.

2. Results and discussion

2.1. Chemistry

The application of ultrasound in the synthesis of compounds (**2**) and (**3**) is fulfilling some of the goals of "green and sustainable chemistry" as it has some advantages comparing with the traditional thermal methods in terms of reaction times, yields and purity of the products (*16, 17*). The reaction of 4,7-dichloroquinoline and *o*-phenylenediamine in the presence of ethanol under reflux in an ultrasonic bath, for 30 min at 90°C yielded N-(7-chloroquinolin-4-yl)-benzene- 1,2-diamine (**2**) (*cf.* Scheme 1). The synthesized quinoline derivative (**2**) was then

reacted with carbonyl compounds such as acetyl naphthalene and 3,4-dimethoxy acetophenone in ethanol under reflux in an ultrasonic bath, for 30 min at 90°C forming Shiff's bases (*E*)-N^1-(7-chloroquinolin-4-yl)-N^2-(1-(naphthalene -8-yl)ethylidene)-benzene-1,2-diamine (**5**) and 7(*E*)-N^1-(7-chloroquinolin-4-yl)-N^2-(1-(3,4-dimethoxyphenyl)-ethylidene)-benzene-1,2-diamine (**6**), respectively (*cf.* Scheme 2). 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (**3**) was prepared by nucleophilic substitution reaction involving 4,7-dichloroquinoline and thiosemicarbazide. The click reactions were achieved in ethanol under reflux in an ultrasonic bath, for 30 min at 90°C (*cf.* Scheme 1). Compound (**3**) which upon cyclization with ethylacetoacetate in an ultrasonic bath, for 40 min at 90°C gave 3-[(7-chloroquinolin-4-yl)amino]-6-methyl-2-thioxo- 2,5-dihydro pyrimidin-4(3*H*)-one (**9**) in excellent yield, then the reaction of compound (**3**) with carbonyl compounds (acetyl naphthalene and 3,4-dimethoxy acetophenone) in ethanol under reflux in an
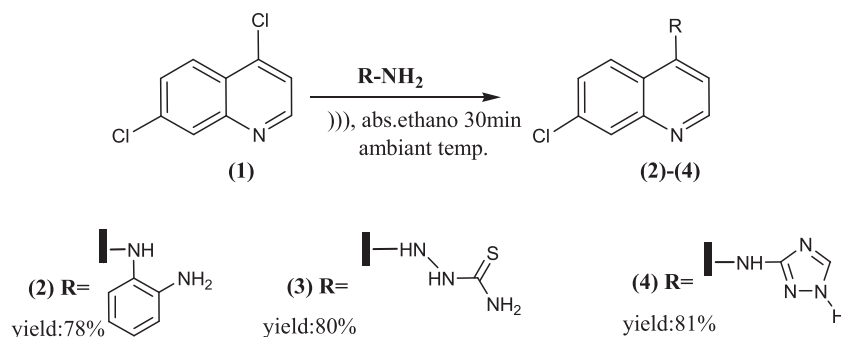
Scheme 1.
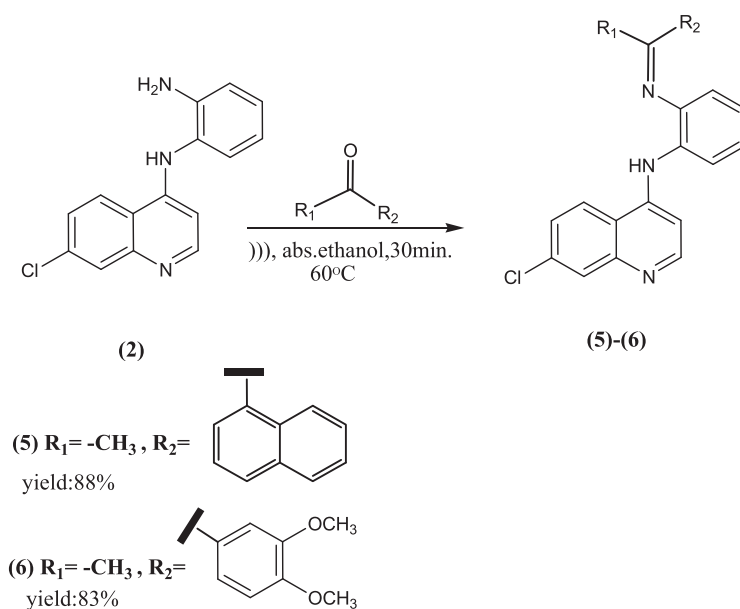
Scheme 2.
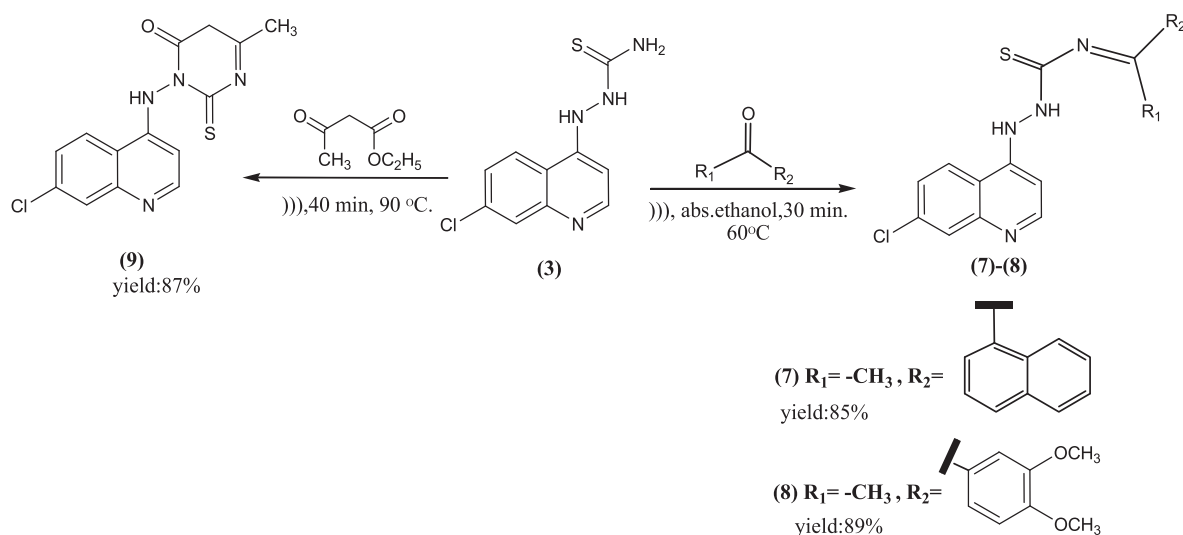
Scheme 3.

ultrasonic bath, for 30 min at 90°C yielded the Shiff's bases (E)-1-(7-chloroquinolin-4-yl) -4-(1-(naphthalen-1-yl)ethylidene)thiosemicarbazide (**7**) and 2-(7-chloroquinolin-4-yl)-N- [(1Z)-1- (3,4-dimethoxyphenyl)ethylidene] hydrazine carbothioamide (**8**), respectively (cf. Scheme 3). Finally, the nucleophilic substitution of 4,7-dichloroquinoline with 3-Amino-1,2,4-triazole in ethanol under reflux in an ultrasonic bath, for 30 min at 90°C gave 7-chloro-N-(1H-1,2,4-triaz-ol-3-yl)-quinolin-4-amine (**4**). The yields for the several click reactions reported here were in the range of 78–89%, synthetic route of the compounds is outlined in Schemes 1, 2 and 3. The structure of the compounds (**2**)–(**9**) was confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectral data.

The first step of the proposed strategy was the synthesis of the key derivatives (**2**)–(**4**) which carried out via nucleophilic substitution on the suitably position of 4-7-dihloroquinoline. It should be noted that the chloroquinoline ring can be considered as the prototype electron poor aromatic system. The electronegative nitrogen substituted on the ring has it's aromaticity disrupted through a strong permanent dipole by the inductive polarization that causes fractional positive charge on the C2 and C4 atoms of the chloroquinoline ring. Therefore, 4-7-dihloroquinoline ring can be pictured as experiencing mesomeric electron withdrawal by the nitrogen atom, and more importantly, experiences additional

inductive withdrawal from the chlorine atom at the C4 (18–21). This means that the chloroquinoline preferably undergoes nucleophilic aromatic substitution via an addition of a nucleophile at C4, followed by the elimination of chlorine atom (SNAE). The present research, in combination with prior studies clearly demonstrates that regioselectivity is often achievable, this regioselectivity is also in agreement with that predicted using the ^{1}H NMR guidelines reported earlier by this group (22). Thus, the chemical shift values for the 7 and 4 positions of quinoline are 7.26 and 8.00, respectively, thereby indicating that C4 should be the more reactive site for SNAE reactions. It is also in agreement with the regioselectivity predicted by the bond dissociation energy calculations reported by Houk and co-workers for the corresponding chloroquinolines (96.2 kcal/Mol for C4 and 97.5 kcal/Mol for C7) (23–25).

2.2. Biological activity

2.2.1. Antimalarial activity

The eight 7-chloroquinoline derivatives were assessed for their in vitro antimalarial action against P. falciparum. Table 1 shows the values of IC_{50} for the vitro antimalarial assay. All newly synthesized compounds showed moderate antimalarial with $IC_{50} < 100$ μM, six of them showed high antimalarial activity with $IC_{50} < 50$ μM in the range of 11.92–79.71 μM. The compounds (**2**), (**3**), (**4**), (**6**), (**8**)

Table 1. The yield of click reaction affording 7-chloroquinoline derivatives (**2**)–(**9**), in virto antimalarial activity (IC_{50} μM) against Plasmodium falciparum.

| Compound	2	3	4	5	6	7	8	9	CQ[a]
Yield	78%	80%	81%	88%	83%	85%	89%	87%	
IC_{50} (μM)	35.29	25.37	42.61	77.60	49.68	79.85	38.71	11.92	0.18

[a]Standard dose (0.01 μM)

and (**9**) showed high activities due to the amination of 4,7-dicloroquinoline with *o*-phenylendiamine, thiosemi-carbazide and 3-Amino-1,2,4-triazole via SNAE, then treated with different carbonyl compounds which increase their activities through the substituents on the amino group. The most active 7-chloroquinoline derivative was the compound (**9**) with an IC_{50} of 11.92 μM that is because this compound contains thioxopyrimidi-none ring, suggesting that its presence could contribute to the activity (*26*). Interestingly, the other active compound is the compound (**2**) with an IC_{50} of 35.29 μM, compound (**3**) with an IC_{50} of 25.37 μM, compound (**4**) with an IC_{50} of 42.61 μM, compound (**6**) with an IC_{50} of 49.68 μM and compound (**8**) with an IC_{50} of 38.71 μM, The significant activity was observed for compounds (**6**) and (**8**) which having two -OCH_3 group on quinoline moiety, the presence of this group could elevate the activity (*27, 28*) where this functional group can do an electron transfer to the protein of the plasmodium para-site that led to the destruction and death of the parasite (*28*). However, compounds (**5**) and (**7**) showed less anti-malarial activity with an $IC_{50} > 50$ μM, the absence of active substituent on the amino group might resulted in less biological activities.
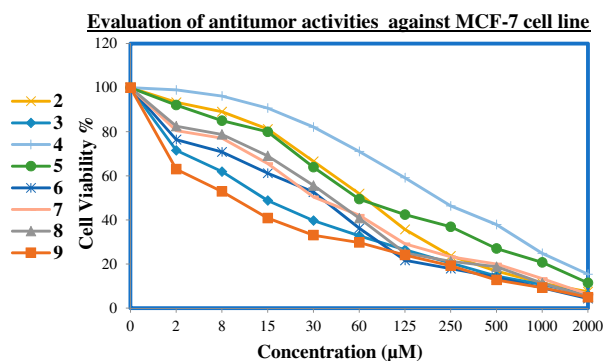
2.2.2. Antitumor activity

Compounds (**2**)–(**9**) were screened for their antitumor activity on three tumor cell lines, **MCF-7** (human breast cancer) cell lines, **HCT-116** (colon carcinoma) cell lines, **Hela** (Cervical carcinoma) cell lines and as a control cell, the human normal liver cell lines **HL-7702** were employed to discern an unspecific of new synthesized compounds. All compounds have been made visible antitumor activity. In general, there is less difference between the antitumor activities of all compounds. Thus, compounds (**3**), (**7**) and (**9**) exerted the highest activities on all cell lines and showed special selectivity toward **MCF-7** cells with IC_{50} 14.68, 14.53 and 7.54 μM, respectively (*cf*. Table 2 and Figures 1–3). The presence of sulfur and nitrogen atoms in the heterocyclic rings in the compounds (**3**) and (**9**) prompts altogether greater selectivity in the direction of **MCF-7** cell lines.
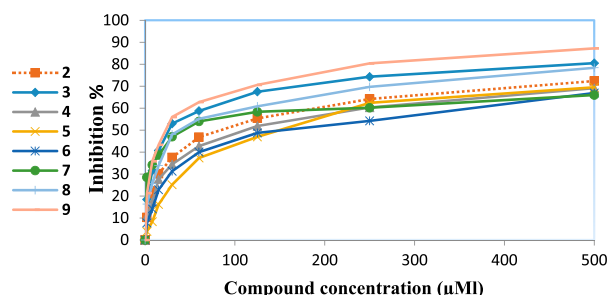
Figure 1. Nonlinear regression analysis from X-axis (inhibition %) to Y-axis (inhibitor concentration in μM), in virto antimalarial activity (IC_{50} μM) against *Plasmodium falciparum*.
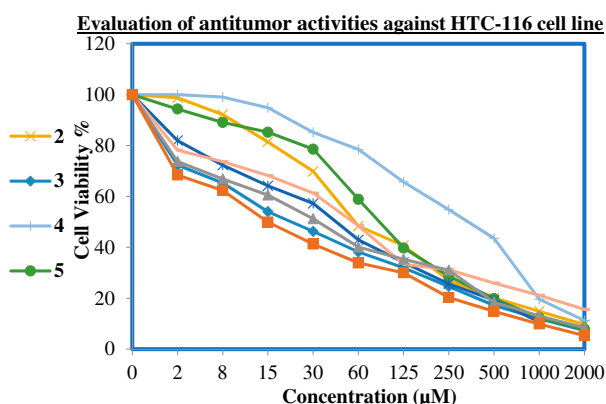
Figure 2. Evaluation of antitumor activities of 7-chloroquinoline derivatives (**2**)–(**9**) against **HTC-116** cell lines.

Figure 3. Evaluation of antitumor activities of 7-chloroquinoline derivatives (**2**)–(**9**) against **MCF-7** cell lines.

Table 2. The 50% inhibitory concentration (IC_{50}) of 7-chloroquinoline derivatives (**2**)–(**9**) against **HELA**, **HTC-116** and **MCF-7** cell lines.

| Compounds | Inhibitory concentration IC_{50} (μM) | | | Unspecific IC_{50} (μM) |
	Hela	MCF-7	HTC-116	Human normal liver **HL-7702**
2	100.05 ± 1.02	61.71 ± 1.02	55.71 ± 1.01	123.05 ± 1.02
3	50.03 ± 0.92	14.68 ± 1.02	23.49 ± 1.02	279.94 ± 1.02
4	372.45 ± 0.23	218.76 ± 1.01	364.36 ± 1.04	328.56 ± 1.04
5	113.94 ± 1.04	36.34 ± 1.04	54.29 ± 1.01	61.97 ± 1.01
6	64.76 ± 1.03	20.91 ± 1.01	27.26 ± 1.04	298.64 ± 0.84
7	74.35 ± 1.01	15.53 ± 0.84	72.84 ± 1.01	280.86 ± 1.04
8	51.67 ± 1.04	27.58 ± 1.03	51.68 ± 1.01	258.23 ± 1.02
9	21.41 ± 0.95	7.54 ± 1.04	21.41 ± 1.05	346.14 ± 1.04

The elucidation for this could be either in the ability of these atoms to communicate (bind) with cellular macromolecules (proteins and/or DNA) and to stimulate a similar reaction in tumor cells (e.g. DNA-damage response), or the most mindful usefulness for this activity is the thiosemicarbazide moiety with the quinoline ring. 7-Chloroquinoline derivatives (3), (6) and (9) have a more pronounced antitumor effect on **HCT-116** (colon carcinoma) cells with IC_{50} 23.39, 27.26 and 21.41 μM, respectively. All compounds showed mild activity toward **Hela** cell lines except (3), (8) and (9) which has IC_{50} 50.03, 51.67 and 21.41 μM, respectively. On the other hand, the compound (4) presented less antitumor activity against all cell lines. Regarding the on the human normal liver cell line **HL-7702**, in general, all newly synthesized compounds are not toxic to normal human cells, however, the low activity was detected for the compound (9) IC_{50} 346.14 μM. The compound (5) was the most toxic compound showing IC_{50} 61.97 μM.

2.2.3. Antibacterial activity

The study examined the antibacterial information of all newly synthesized compounds (2)–(9) which showed a very good to moderately good activities towards different strains of microorganisms (12.5 ± 0.63–23.8 ± 1.5) (Table 3). The compounds (3), (4), (7) and (9) showed relatively perfect activity with all the bacterial strains. The best activity is attributed to the presence of pharmacologically active groups at position 4 of quinoline, –NH– and triazole ring attached to quinoline ring. Introduction of thiosemicarbazide group such as NH-NH –CS–NH$_2$ to the ring quinoline at position 4 promoted activity while the introduction of o-phenylene diamine derivatives group at quinoline caused a reduction in activity versus most of the bacterial strains. Compounds (7) and (9) demonstrated greater activity against the gram-negative bacterial species *Staphylococcus aureus*, however, compounds (4) and (8) exhibit a good action versus the gram-negative bacterial species *Bacillus subtilis*. Fulfillment of gram-negative antibacterial, compounds (4) and (9) demonstrated amazing activity against *Salmonella typhimurium*, while compounds (7) and (8) showed very good activities against *Escherichia coli*. DMSO was used as a control solvent and Ampicillin and Ciprofloxacin, as reference drugs.

The compounds showed relatively very good activities against all the fungal strains. These compounds include biologically active groups, –NH– and triazole ring attached to quinoline ring. The compounds (3), (4), (7) and (9) exhibited higher activity against all fungal strains. DMSO was used as a control solvent and Amphotericin B, as a reference drug. It is worth noting that the presence of hetero rings at the fourth position of the quinoline system significantly increases the biological effect against fungal strains.

3. Materials and methods

3.1. Chemical syntheses- general

Melting points were measured with a Gallen Kamp melting point apparatus. Silica-gel-coated aluminum plates used to test the purity of the compounds. Infrared spectra (λ-cm^{-1}) were recorded on Bruker Vector (Germany) and on Mattson FT-IR 1000 (Taibah University, Saudi Arabia), using KBr disks. ^{1}H NMR spectra were recorded on Gemini 300 MHz, C^{13} NMR spectrometer, in DMSO-d$_6$ using dimethyl sulfoxide as a solvent and tetramethylsilane (TMS) as an internal standard (Chemical shift in δ, ppm); ^{13}C NMR spectra were recorded on Gemini 50 MHz NMR spectrometer. Mass spectra were measured on GCQ Finnigan MAT and Elemental analyses were performed at the microanalytical Center, Cairo University, Giza, Egypt. Biological activity was determined in a laboratory by the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. All the chemicals were purchased from Sigma–Aldrich.

3.1.1. General procedure for synthesis 1-(7-chloroquinolin-4-yl) derivatives (2)–(4)

(0.01 Mol) 4,7-dichloroquinoline (1) was mixed with (0.01 Mol) of the appropriate amine, o-phenylenediamine, thiosemicabazide and 3-amino-1,2,4-triazole in ethanol (15 ml) and refluxed in an ultrasonic bath for 30 min at ambient temperature. TLC monitoring was used to ensure the completion of the reaction. Then we added CH$_3$Cl (150 ml) as an organic solvent, then washed the mixture with NaOH (1N, 150 ml) to give an organic layer and aqueous layer. The resulting crude poured into ice water to produce compounds (2)–(4) and the solid formed was collected by filtration and dried.

3.1.2. General procedure for synthesis Schiff bases (5)–(8), (0.01 Mol) of a compound (2) or (3)

(0.01 Mol) of carbonyl compounds as acetyl naphthalene, 3,4-dimethoxy acetophenone, in 20 ml absolute ethyl alcohol were refluxed in an ultrasonic bath, for 30 min at 90℃, until TLC analysis showed the complete consumption of 1-(7-chloroquinolin-4-yl) derivatives by using a proper eluent. By concentrating the filtered it gave compounds (5)–(8) which purified by preparative column chromatography to give the desired product in good to excellent yields.

3.1.3. General procedure for synthesis 3-(7-chloroquinolin-4-ylamino)-tetrahydro-6-methyl-2-thioxopyrimidin-4(1H)-one (9)

(0.01 Mol) of a compound (**3**) and (0.01 Mol) of ethyl acetoacetate was refluxed in an ultrasonic bath, for 40 min at 90°C, to give brown crystals which recrystallized from ethyl alcohol to give the product (**9**).

N^1-(7-chloroquinolin-4-yl)benzene-1,2-diamine (**2**): Show the following data; a brown precipitate which was washed with ether and dried. Yield: 88%; mp 213–215°C, R$_f$ 0.54(1:6 EtOAc–hexane); IR(KBr) cm^{-1}: 3490–3416 (NH$_2$), 3162 (NH), 1607 (C=N), 1569 (NH bend), 752 (C-Cl); ^{1}H NMR(300 MHz, DMSO-d$_6$) δ 8.89 (d, J = 5.39 Hz, 1H, N=CH, quinoline), 8.59 (s, 1H, ArC- NH), 8.43(d, J = 5.48 Hz, 1H, Ar- H), 8.32(d, J = 8.48 Hz, 1H, Ar-H), 8.21(d, J = 8.43 Hz, 1H, Ar- H), 8.17(s, 1H, Ar- H), 7.84 (d, J = 8.41 Hz, 1H, Ar- H), 7.81(d, J = 8.41 Hz, 1H, Ar- H), 7.79(dd, J = 7.22 Hz ,J = 8.32 Hz, 1H, Ar- H), 7.74(dd, J = 7.02 Hz ,J = 8.34 Hz, 1H, Ar- H), 6.11 (s, 2H, NH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 159.42, 157.52, 156.71, 150,03, 144.82, 143.14, 138.60, 136.35, 130.62, 127.92, 127.85, 127.03, 125,50, 120.05, 90.13. Mass spectra (M±) at m/z 269. Anal. Calcd for C$_{15}$H$_{12}$N$_3$Cl: C, 66.79; H, 4.48; N, 15.58. Found: C, 66.82; H, 4.67; N, 15.67%.

2-(7-chloroquinolin-4-yl)thiosemicarbazide (**3**): Show the following data; a yellow precipitate which was washed with ether and dried. Yield: 80%; mp 278–280°C, R$_f$ 0.38 (1:2 EtOAc-petroleum ether); IR (KBr) cm^{-1} :3390–3266 (NH$_2$), 3150 (NH), 1615 (C = N), 1585 (NH bend), 1235 (C–N), 1212 (C=S), 758 (C-Cl); ^{1}H NMR(300 MHz, DMSO-d$_6$) δ 8.89(d, J = 5.48 Hz, 1H,N=CH, quinoline), 8.57(d, J = 8.48 Hz, 1H, Ar- H), 8.54(s, 1H, Ar- H), 8.26(d, J = 8.43 Hz, 1H, Ar- H), 8.20 (s, 2H, NH$_2$), 8.10 (s, 1H, ArC-NH), 7.93(d, J = 5.42 Hz, 1H, Ar- H), 7.86 (s, 1H, NH); ^{13}C NMR (50 MHz, CDCl$_3$) 181.22, 158.22, 155.72, 149.02, 148.14, 138.50, 128.42, 127.65, 120.03, 18.34. Mass spectra (M±) at m/z 252. Anal. Calcd for C$_{10}$H$_9$ClN$_4$S: C,47.53; H, 3.59; N, 22.17.Found: C, 47.33; H, 3.52; N, 22.09%.

7-chloro-N-(1H-1,2,4-triazol-3-yl)quinolin-4-amine (**4**): Show the following data; a white precipitate which was washed with ether and dried. Yield: 81%; mp 170–172°C, R$_f$ 0.46(1:2 EtOAc-petroleum ether); IR (KBr) cm^{-1}: 3165 (NH), 1621 (C=N), 1579 (NH bend), 764(C-Cl) ; ^{1}H NMR(300 MHz, DMSO-d$_6$):δ 8.89 (d, J = 5.29 Hz, H, N=CH, quinoline), 8.88 (s, H, NH triazole), 8.25 (s, H, ArC-NH), 8.22(d, J = 8.35 Hz, 1H, Ar- H), 8.19(s, 1H, Ar-H), 8.18 (CH triazole), 7.83(d, J = 8.38 Hz, 1H, Ar- H), 7.79 (d, J = 8.43 Hz, 1H, Ar- H); ^{13}C NMR (50 MHz, CDCl$_3$) δ 159.41, 158.91, 157.32, 155.12, 139.62, 133.14, 131.80, 120.73, 118.33, 116.37, 99.89. Mass spectra (M±) at m/z 245. Anal. Calcd for C$_{11}$H$_8$N$_5$Cl: C, 53.78; H, 3.28; N, 28.51. Found: C, 53.57; H, 3.32; N, 28.41%.

(E)-N^1-(7-chloroquinolin-4-yl)-N^2-(1-(naphthalen-8-yl)ethylidene)-benzene-1,2-diamine (**5**): Show the following data; a yellow oil which purified by column chromatography by using ethyl acetate and hexane (3:1) to give yellow crystals. Yield: 88%; mp 237–239°C, R$_f$ 0.38 (3:1 EtOAc–hexane); IR (KBr) (cm^{-1}) 3184 (NH), 3020 (Ar.C–H), 2941 (CH$_3$, str.), 1626 (C=N); ^{1}H NMR(300 MHz, DMSO-d$_6$) δ 8.85 (d, J = 5.45 Hz, 1H, N=CH, quinoline), 8.16(d, J = 8.34 Hz, 1H, Ar- H), 7.89(d, J = 8.46 Hz, 1H, Ar-H), 7.88(d, J = 8.43 Hz, 1H, Ar- H), 7.81(d, J = 8.41 Hz, 1H, Ar- H), 7.79(s, 1H, Ar- H), 7.77(dd, J = 7.42 Hz, J = 8.42 Hz, 1H, Ar- H), 7.75(dd, J = 8.42 Hz,J = 7.22 Hz 1H, Ar- H), 7.70 (s, 1H, ArC- NH), 7.35–7.45 (m,7H,Ar-H), 6.47 (d, J = 5.39 Hz, 1H, Ar- H), 2.66 (s, 3H, CH$_3$); ^{13}C NMR (50 MHz CDCl$_3$) δ 160.27, 155.17, 150.43, 145.32, 142.34, 140.26, 137.54, 135.24, 133.78, 132.11, 130.63, 130.56, 130.45, 128.15, 126.87, 126.48, 126.25, 125.73, 125.30, 124.70, 124.10, 123.67, 123.11, 122.03, 117.40, 113.52, 94.45. Mass spectra (M±) at m/z 421. Anal. Calcd for C$_{27}$H$_{20}$N$_3$Cl: C, 76.86; H, 4.78; N, 9.96. Found: C, 76.91; H, 4.52; N, 9.88%.

(E)-N^1-(7-chloroquinolin-4-yl)-N^2-(1-(3,4-dimethoxyphenyl)-ethylidene)-benzene-1,2-diamine (**6**): Show the following data; a yellowish red precipitate which purified by column chromatography by using ethyl acetate and CHCl$_3$ (3:1) to give yellow crystals. Yield: 83%; mp 249–251°C, R$_f$ 0.48(3:1 EtOAc- CHCl$_3$); IR (KBr) (cm^{-1}) :3162 (NH), 3041 (Ar.C–H), 2952 (CH$_3$, str.), 1615 (C=N), 1268 (O-CH$_3$); ^{1}H NMR(300 MHz, DMSO-d$_6$) δ 8.89 (d, J = 5.37 Hz, 1H, N=CH, quinoline), 8.32 (s, 1H, ArC- NH), 8.29(d, J = 5.42 Hz, 1H, Ar- H), 8.17(d, J = 8.48 Hz, 1H, Ar-H), 7.91(d, J = 8.43 Hz, 1H, Ar- H), 7.88 (s, 1H, Ar- H), 7.79 (d, J = 8.41 Hz, 1H, Ar- H), 7.77(d, J = 8.38 Hz, 1H, Ar- H), 7.61 (dd, J = 7.22 Hz ,J = 8.32 Hz, 1H, Ar- H), 7.51(dd, J = 7.02 Hz ,J = 8.34 Hz, 1H, Ar- H), 7.41 (s, 1H, Ar- H), 7.36 (d, J = 8.29 Hz, 1H, Ar- H), 7.33 (d, J = 8.33 Hz, 1H, Ar- H), 3.81–3.84 (s, 6H, 2OCH$_3$), 2.50 (s, 3H, CH$_3$); ^{13}C NMR (50 MHz, CDCl$_3$) 169.41, 154.82, 154.31, 153.12, 151.76, 149.09, 145.21, 143.54, 139.91, 133.42,1 31.11, 127.53, 124.2, 123.90, 123.05, 122.17, 119.83, 117.56, 116.98, 116.03, 111.08, 96.15, 58.16, 55.66, 30.60. Mass spectra (M±) at m/z 431. Anal. Calcd for C$_{25}$H$_{22}$N$_3$ClO$_2$: C, 69.52; H, 5.13; N, 9.73. Found: C, 69.66; H, 5.09; N, 9.69%.

(E)-1-(7-chloroquinolin-4-yl)-4-(1-(naphthalen-1-yl)ethylidene)thiosemicarbazide (**7**): Show the following data; a brown oil which purified by column chromatography by using ethyl acetate and CHCl$_3$ (3:1) to give yellow crystals. Yield: 85%; mp 210–212°C, R$_f$ 0.42(3:1 EtOAc- CHCl$_3$); IR (KBr) (cm^{-1}) 3172 (NH), 3025 (Ar.C–H), 2943 (CH$_3$, str.), 1623(C=N), 1580 (NH bend), 1224 (C=S); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 2.71 (s, 3H, CH$_3$), δ8.87 (d, J = 5.25 Hz, 1H,N=CH, quinoline), 8.57 (d, J = 8.17 Hz, 1H,

Ar- H), 8.43 (s, 1H, Ar- H), 8.31(d, $J = 8.43$ Hz, 1H, Ar- H), 8.16 (s, 1H, NH), 8.00 (s, 1H, NHCS), 7.29–7.51 (m, 7H, Ar-H), 6.64(d, $J = 5.29$ Hz, 1H, Ar- H); ^{13}C NMR (50 MHz, CDCl$_3$) δ 187.57, 162.64, 151.34, 149.02, 145.84, 136.91, 135.89, 134.19, 131.45, 129.43, 128.07, 127.73, 126.59, 126.32, 125.06, 122.76, 122.21, 121.06, 114.26, 109.44, 98.36, 26.71. Mass spectra (M±) at m/z 404. Anal. Calcd for C$_{22}$H$_{17}$N$_4$ClS: C, 65.26; H, 4.23; N, 13.84. Found: C, 65.33; H, 4.17; N, 13.88%.

(*E*)-1-(7-chloroquinolin-4-yl)-4-(1-(3,4-dimethoxyphe-nyl)ethylidene)thiosemicarbazide (**8**): Show the following data; a brown precipitate which was purified by column chromatography by using ethyl acetate and CHCl$_3$ (3:1) to give a brown crystal. Yield: 89%; mp 251–253°C, R$_f$ 0.45(3:1 EtOAc- CHCl$_3$); IR(KBr) cm^{-1} : 3165 (NH), 2950 (CH$_3$, str.), 1618 (C=N), 1585 (NH bend), 1266 (O-CH$_3$), 1230 (C–N), 1221 (C=S), 750 (C-Cl); ^{1}H NMR(300 MHz, DMSO-d$_6$) δ 9.23 (d, $J = 5.30$ Hz, 1H, N=CH, quinoline), 8.91 (s, 1H, ArC- NH), 8.31(d, $J = 8.40$ Hz, 1H, Ar- H), 8.23 (s, 1H, NHCS), 8.26 (d, $J = 8.43$ Hz, 1H, Ar- H), 8.19 (s, 1H, Ar- H), 7.90 (d, $J = 5.38$ Hz, 1H, Ar- H), 7.87 (s, 1H, Ar- H), 7.85 (d, $J = 8.39$ Hz, 1H, Ar- H), 7.81(d, $J = 8.36$ Hz, 1H, Ar- H), 3.29 (s, 6H, 2OCH$_3$), 2.08 (s, 3H, CH$_3$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 182.95, 154.43, 153.82, 149.56, 148.71, 147.12, 137.31, 128.05, 127.51, 121.26, 119.50, 116.74, 115.06, 110.44, 109.91, 108.54, 103.38, 50.87, 50.72, 25.80. Mass spectra (M±) at m/z 414. Anal. Calcd for C$_{20}$H$_{19}$N$_4$ClO$_2$S: C, 57.90; H, 4.46; N, 13.50; Found: C, 57.73; H, 4.32; N, 13.62%.

3-(7-chloroquinolin-4-ylamino)-tetrahydro-6-methyl-2-thioxopyrimidin-4(1H)-one (**9**): Show the following data; a brown crystals which was washed with ether and dried. Yield: 87%; mp 250–252°C R$_f$ 0.48(1:2 EtOAc-petroleum ether); IR (KBr) cm^{-1}: 3110 (NH), 1674 (C=O), 1618 (C=N), 1587 (NH bend), 1237 (C–N), 1211 (C=S), 760(C-Cl) ; ^{1}H NMR(300 MHz, DMSO-d$_6$):δ 8.20 (d, $J = 5.38$ Hz, 1HN=CH quinoline) 8.10 (s, 1H, NH);, 7.85 (d, $J = 8.32$ Hz, 1H, Ar- H), 7.82 (s, 1H, Ar- H), 7.18 (d, $J = 5.38$ Hz, 1H,

Ar- H), 7.15 (d, $J = 5.38$ Hz, 1H, Ar- H), 2.50 (s, 2H, CH$_2$), 2.17 (s, 3H, CH$_3$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 181.36, 166.23, 156.96, 151.13, 149.15, 141.30, 138.57, 122.23, 119.52, 118.17, 115.31, 113.80, 91.21, 48.79. Mass spectra (M±) at m/z 318. Anal. Calcd.for C$_{14}$H$_{11}$N$_4$ClOS: C, 52.75; H, 3.48; N, 17.58. Found: C, 52.91; H, 3.32; N, 17.71%.

3.2. Biological assays

3.2.1. Determination of antimalarial activity
Cultures of *P. falciparum* were measured according to a protocol from Molony et al. (*29*) and Trager and Williams (*30*). The percentage of Inhibition was studied the 50% inhibitory concentrations (IC$_{50}$) was measured and the data got from the inhibitor-dependent concentration growth curves were registered into plots with nonlinear retreating analysis from *y*-axis (inhibition %) to *x*-axis (inhibitor concentration) (*31*) (*cf.* Table 1, Figure 4). *P. fal-ciparum* isolate NF54 was maintained in continuous culture with gentamicin (40 µg/mL) in Petri dishes (5 cm in diameter) with a gaseous phase of 90% N$_2$, 5% O$_2$ and 5% CO$_2$, according to a protocol from Moloney et al. (*29*) and Trager and Williams (*30*). *P. falciparum* para-sites were cultured in human erythrocytes (blood group A$^+$ at 10% (v/v) hematocrit) in RPMI 1640 medium (Sigma) supplemented with 25 mM HEPES, 20 mM sodium bicarbonate, and 10% heat-inactivated human A$^+$ plasma. The culture was routinely monitored through Geimsa staining of the thin blood smears. The parasitemia of the infected erythrocytes was determined in Giemsa-stained smears by light microscopy. Parasite-mias and morphological changes detected in the cultures were scored visually with a 100-fold oil-immer-sion objective, counting at least 1000 erythrocytes to determine the percentage of the infected erythrocytes (*32*).

Antimalarial activity assay: The experiments were per-formed in 96-well culture plates (Nunc); compounds were tested at two-fold dilutions in a dose-titration range of 500 µM to 2 µM. One hundred microliters of infected human red blood cell suspension (1% parasite-mia, 4% hematocrit), with more than 90% of ring forms, were added to each well containing 100 ml of extracts pre-diluted in RPMI-1640. Test plates were incubated for 48 h. Parasite multiplication was determined microscopi-cally after Giemsa staining and expressed as a percentage of the controls without test compounds. A drug-free control (methanol/water 50:50% v,v) was used in all experiments and CQ (0.01 µM) was used as the standard reference drug. Parasitemia and stage distribution were estimated as triplicates daily from Giemsa-stained smears by counting 1000 erythrocytes (*33*).
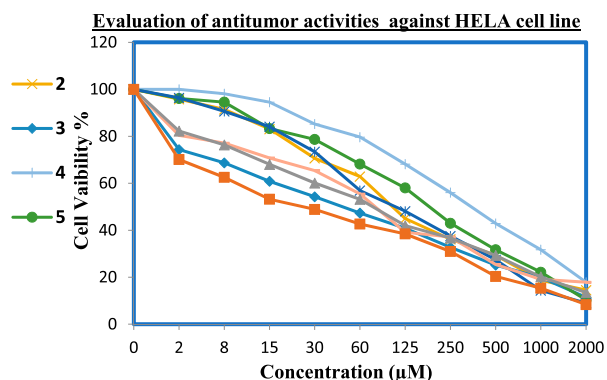
Figure 4. Evaluation of antitumor activities of 7-chloroquinoline derivatives **(2)-(9)** against **HELA** cell lines.

Table 3. Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinical pathogenic microorganisms using (5 mg/ml) concentration of samples.

Compounds	2	3	4	5	6	7	8	9	St.
Tested microorganisms									
FUNGI									*Amphotericin B*
Aspergillus fumigatus (RCMB 02568)	16.8 ± 0.63	13.3 ± 0.58	18.4 ± 0.58	13.1 ± 0.81	16.3 ± 0.19	20.17 ± 0.82	15.8 ± 0.73	19.2 ± 0.64	23.7 ± 1.2
Candida albicans (RCMB 05036)	14.4 ± 0.58	20.2 ± 1.5	16.9 ± 0.63	14.1 ± 0.66	13.6 ± 1.2	23.8 ± 0.95	16.4 ± 1.4	21.1 ± 0.32	25.4 ± 0.58
Gram Positive Bacteria									*Ampicillin*
Staphylococcus aureus (RCMB 010028)	16.9 ± 1.5	19.6 ± 0.63	19.1 ± 1.2	12.9 ± 1.3	17.3 ± 0.63	20.09 ± 0.63	13.9 ± 1.1	21.41 ± 0.92	23.8 ± 1.2
Bacillis subtilis (RCMB 010067)	16.2 ± 1.2	17.9 ± 0.28	22.2 ± 1.5	13.9 ± 15	15.3 ± 1.5	17.1 ± 00.66	23.2 ± 0.78	18.3 ± 1.1	27.4 ± 0.72
Gram-negative Bacteria									*Ciprofloxacin*
Salmonella typhimurium (RCMB 010072)	18.3 ± 0.63	17.3 ± 1.2	20.6 ± 1.2	18.1 ± 1.5	19.2 ± 0.58	19.6 ± 0.94	17.3 ± 0.83	20.2 ± 1.5	20.6 ± 1.2
Escherichia coli (RCMB 010052)	12.5 ± 0.63	19.6 ± 0.44	15.5 ± 1.2	15.2 ± 0.95	20.06 ± 0.58	20.5 ± 1.1	22.3 ± 0.85	20.2 ± 0.72	23.4 ± 0.63

3.2.2. Determination of antitumor activity against MCF-7, HCT-116 and Hela cell lines

The antitumor activities of all new synthesized 7-chloroquinoline derivatives were tested for three cancer cell lines **MCF-7 cells** (human breast cancer), **HCT-116** (colon carcinoma) and **Hela** (Cervical carcinoma). As control cell, the human normal liver cell line **HL-7702** was employed to discern an unspecific cytotoxicity of newly synthesized 7-chloroquinoline derivatives. All cell lines were gotten from the VACSERA Tissue Culture Unit. The effect of new compounds on the cell was calculated and the microplate reader (SunRise, TECAN, Inc, U.S.A.) was utilized to gauge the optical density and to determine the number of viable cells and the percentage of viability which calculated as $[1-(ODt/ODc)] \times 100\%$ where ODt is the mean Optical Density of wells dealt with the tested sample and ODc is the mean Optical Density of untreated Cells (*34, 35*).

3.2.3. Determination of antimicrobial activity

The antimicrobial activity was measured for the newly synthesized compounds (**2**)–(**9**). Each microbial strain of culture collection was given by the RCMB, Al-Azhar University, Cairo, Egypt. The antimicrobial profile was tested against two Gram-positive bacteria species (*B. subtilis*, *S. aureus*), two Gram- negative bacterial species (*E. coli*, *S. typhimurium*), two fungi (*Aspergillus fumigatus* and *Candida albicans*) utilizing an adjusted well dissemination strategy (*36–38*) .

4. Conclusions

The present study describes the synthesis and antimalarials, antimicrobial, and the anticancer activity of a novel series of 7-chloroquinoline derivatives via click chemistry by using ultrasound irradiation energy source. All the compounds exhibit potent antimalarial activity in vitro. Further, the synthesized compounds (**2**)–(**9**) were screened for antibacterial activity against two Gram-positive bacteria species (*B. subtilis*, *S. aureus*), two Gram-negative bacterial species (*E. coli*, *S. typhimurium*), two fungi (*A. fumigatus* and *C. albicans*) two pathogenic strains (*E. coli* and *B. subtilis*). The results appear clearly that the most of the new compounds have shown very good biological activities.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Asmaa Aboelnaga was born in Cairo, Egypt. She received a Ph.D. degree from the Ain Shams University, Egypt; she

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