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Original research paper

Design, synthesis and potential anti-proliferative activity of some novel 4-aminoquinoline derivatives

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Novel nineteen compounds based on a 4-aminoquinoline scaffold were designed and synthesized as potential anti-proliferative agents. The new compounds were *N*-substituted at the 4-position by aryl or heteroaryl (**1-9**), quinolin-3-yl (**10**), 2-methylquinolin-3-yl (**11**), thiazol-2-yl (**12**), and dapsone moieties (**13**, **14** and **18**). Bis-compounds **15**, **16** and **19** were also synthesized to assess their biological activity. All the newly synthesized comounds were tested for *in vitro* antiproliferative activity against the MCF-7 breast cancer cell line. Seventeen of the novel compounds showed higher activity than the reference drug doxorubicin. The corresponding 7-(trifluoromethyl)-*N*-(3,4,5-trimethoxyphenyl)quinolin-4-amine **1**, *N*-(7-(trifluoromethyl)quinolin-4-yl)quinolin-3-amine (**10**), 2-methyl-*N*-(7-trifluorome-thyl)quinolin-4-yl) quinolin-3-amine (**11**) and *N*-(4-(4-aminophenylsulfonyl) phenyl)-7-chloroquinolin-4-amine (**13**) were almost twice to thrice as potent as doxorubicin. Biological screening of the tested compounds could offer an encouraging framework in this field that may lead to the discovery of potent anticancer agents.

Keywords: 4-aminoquinolines, bis-compounds, dapsone, antiproliferative activity

Nitrogen containing heterocycles are indispensable structural units in medicinal chemistry. Among various heterocyclic compounds, quinolines (1, 2) occur predominantely in nature because of their stability and ease of generation. They exhibit a multitude of biological utilities, such as anticancer (3), antioxidant (4), anti-proliferative (5), and anti-inflammatory properties (6). Since the discovery of the chemo-sensitisation of the multi-drug resistance (MDR) cancer cell lines by chloroquine **I** (7) and primaquine **II** (8) in the 1980s (Fig. 1), little work has been done to exploit these antimalarial agents as effective agents in the management of cancer. Also, several adamantane, morpholine, pyrimidine, triazine, quinoline and benzothiazole derivatives were associated with anticancer (9) and antimicrobial (10) activities. In addition, the corresponding biphenylsulfone derivatives

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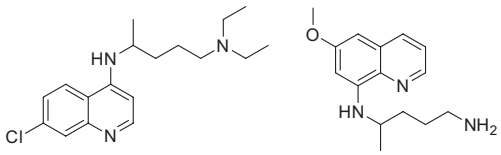
were reported to possess significant anticancer activity (11). In view of the above mentioned findings and as a part of our research efforts to explore novel anti-proliferative heterocyclic compounds (12–18), we have synthesized a new series of 4-aminoquinoline derivatives bearing a number of the biologically active moieties mentioned above, as analogues to compounds **I** and **II**. Anti-proliferative activity of the synthesized compounds was evaluated against the human breast cancer cell line MCF-7.



I **II**

Fig. 1. Structures of chloroquine **I** and primaquine **II**.

EXPERIMENTAL

Melting points (uncorrected) were determined in open capillaries on a Gallen Kemp melting point apparatus (Sanyo Gallen Kemp, UK). Precoated silica gel plates (Kieselgel 0.25 mm, 60 F254, Merck, Germany) were used for thin layer chromatography. A developing solvent system of chloroform/methanol (8:2) was used and the spots were detected by ultraviolet light. IR spectra (KBr disc) were recorded using an FT-IR spectrophotometer (Perkin Elmer, USA). ^{1}H NMR spectra were scanned on a NMR spectrophotometer (Bruker AXS Inc., Switzerland) operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All the values were within ± 0.4 % of theoretical values.

All reagents used were of AR grade. The starting materials 4,7-dichloroquinoline and 4-chloro-7-trifluoromethylquinoline were purchased from Sigma (USA) and were directly used for the preparation of target compounds.

Syntheses

Quinoline derivatives 7-(trifluoromethyl)-N-*(3,4,5-trimethoxyphenyl)quinolin-4-amine* (**1**), N-*(3-methylbicyclo[3.3.1]nonan-3-yl)-7-(trifluoromethyl)quinolin-4-amine* (**2**), *7-chloro*-N-*(4-morpholinophenyl)quinolin-4-amine* (**3**), N-*(4-morpholinophenyl)-7-(trifluoromethyl)quinolin-4-amine* (**4**), *5-(7-(trifluoromethyl)quinolin-4-ylamino)pyrimidin-2,4-(1*H,3H*)-dione* (**5**), *1,3-dimethyl-6-(7-(trifluoromethyl)quinolin-4-ylamino)pyrimidin-2,4-(1*H,3H*)-dione* (**6**), N-*(benzo[d] [1,3] dioxol-5-ylmethyl)-7-chloroquinolin-4-amine* (**7**), N-*(benzo[d] [1,3] dioxol-5-ylmethyl)-7-(trifluoromethyl) quinolin-4-amine* (**8**), N-*(5,6-dimethyl-1,2,4-triazin-3-yl)-7-(trifuoloromethyl) quinolin-4-amine* (**9**), N-*(7-(trifluoromethyl)quinolin-4-yl)quinolin-3-amine* (**10**), *2-methyl*-N-*(7-trifluoromethyl) quinolin-4-yl)quinolin-3-amine* (**11**), *6-methoxy*-N-*(7-(trifluoromethyl) quinolin-4-yl) benzo[*d*]thiazol-2-amine* (**12**). *General procedure* – A mixture of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline (0.01 mol) and the appropriate amine (0.01 mol) in dry

DMF (20 mL) was refluxed for 18 h. The reaction mixture was poured onto ice/water and the obtained solid was recrystallized from dioxane to give **1–12**, respectively (Table I).

N-*(4-(4-aminophenylsulfonyl) phenyl)-7-chloroquinolin-4-amine* (**13**) N-*(4-(4-aminophenyl-sulfonyl) phenyl-7-(trifluoromethyl)quinolin-4-amine* (**14**). – A mixture of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline (0.01 mol) and dapsone (2.28 g, 0.01 mol) in DMF (20 mL) was refluxed for 24 h. The obtained solid was recrystallized from dioxane to give **13** and **14**, respectively (Table I).

N,N′-*(4,4′-sulfonylbis(4,1-phenylene)bis(7-chloroquinolin-4-amine)* (**15**), N,N′-*(4,4′-sulfonylbis(4,1-phenylene)bis(7-(trifluoromethyl)quinolin-4-amine)* (**16**). – A mixture of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline (0.02 mol) and dapsone (2.28 g,

Table I. Physicochemical and analytical data of the newly synthesized compounds

Compd.	Formula (M_r)	M. p. (°C)	Yield (%)	Analysis (calcd./found) (%)		
				C	H	N
1	$C_{19}H_{17}F_3N_2O_3$ (378.35)	213.3	88	60.32/60.64	4.53/4.19	7.40/7.76
2	$C_{20}H_{21}F_3N_2$ (346.41)	220.2	76	68.95/68.68	6.65/6.39	8.04/8.31
3	$C_{19}H_{18}ClN_3O$ (339.82)	326.6	81	67.15/67.47	5.34/5.14	12.37/12.66
4	$C_{20}H_{18}F_3N_3O$ (373.37)	286.0	77	64.34/64.63	4.86/4.58	11.25/11.59
5	$C_{14}H_9F_3N_4O_2$ (322.24)	> 350	69	52.18/52.50	2.82/2.60	17.39/17.14
6	$C_{16}H_{13}F_3N_4O_2$ (350.30))	242.6	73	54.86/54.59	3.74/3.48	15.99/15.66
7	$C_{17}H_{13}ClN_2O_2$ (312.75)	> 350	89	65.29/65.56	4.19/4.49	8.96/8.61
8	$C_{18}H_{13}F_3N_2O_2$ (346.30)	> 350	82	62.43/62.12	3.78/3.48	8.09/8.31
9	$C_{15}H_{12}F_3N_5$ (319.28)	> 350	59	56.43/56.71	3.79/3.58	21.93/21.66
10	$C_{19}H_{12}F_3N_3$ (339.31)	251.2	61	67.25/67.55	3.56/3.19	12.38/12.65
11	$C_{20}H_{14}F_3N_3$ (353.34)	68.8	59	67.98/67.69	3.99/3.70	11.89/11.62
12	$C_{19}H_{14}F_3N_3OS$ (389.08)	208.0	71	58.60/58.35	3.62/3.29	10.79/10.48
13	$C_{12}H_{16}ClN_3O_2S$ (409.89)	136.8	65	61.53/61.18	3.93/3.70	10.25/10.56
14	$C_{22}H_{16}F_3N_3O_2S$ (443.44)	118.1	71	59.59/59.20	3.64/3.28	9.48/9.76
15	$C_{30}H_{20}Cl_2N_4O_2S$ (571.48)	340.1	55	63.05/63.29	3.53/3.19	9.80/9.51
16	$C_{32}H_{20}F_6N_4O_2S$ (638.58)	148.6	61	60.19/60.50	3.16/3.48	8.77/8.45
17	$C_{10}H_5ClN_2S$ (220.68)	114.7	94	54.43/54.67	2.28/2.60	12.69/12.44
18	$C_{22}H_{17}ClN_4O_2S_2$ (468.98)	159.6	59	56.34/56.61	3.65/3.26	11.95/11.69
19	$C_{32}H_{22}Cl_2N_6O_2S_3$ (689.66)	258.9	55	55.73/55.48	3.22/3.50	12.15/12.49

0.01 mol) in DMF (20 mL) was refluxed for 18 h. The obtained solid was recrystallized from ethanol to give **15** and **16**, respectively (Table I).

7-Chloro-4-isothiocyanatoquinoline (**17**). – A mixture of 4,7-dichloroquinoline (1.98 g, 0.01 mol) and ammonium thiocyanate (1.52 g, 0.01 mol) in dry acetone (20 mL) was refluxed for 1 h. The reaction mixture was cooled, poured onto ice/water and the obtained solid was recrystallized from dioxane to give **17** (Table I).

N-(4-(4-aminophenylsulfonyl) phenyl)-N-(7-chloroquinolin-4-yl)carbamimidothioic acid (**18**). – A mixture of **17** (2.20 g, 0.01 mol) and dapsone (2.28 g, 0.01 mol) in DMF (20 mL) containing a catalytic amount of triethylamine was refluxed for 22 h. The obtained solid was recrystallized from acetic acid to give **18** (Table I).

N,N-(4,4′-sulfonylbis(4,1-phenylene)bis(N′-(7-chloroquinolin-4-yl)carbamimido-thioic acid (**19**). – A mixture of **17** (4.4 g, 0.02 mol) and dapsone (2.28 g, 0.01 mol) in DMF (20 mL) containing 3 drops of triethylamine was refluxed for 24 h. The obtained solid was recrystallized from dioxane to give **19** (Table I).

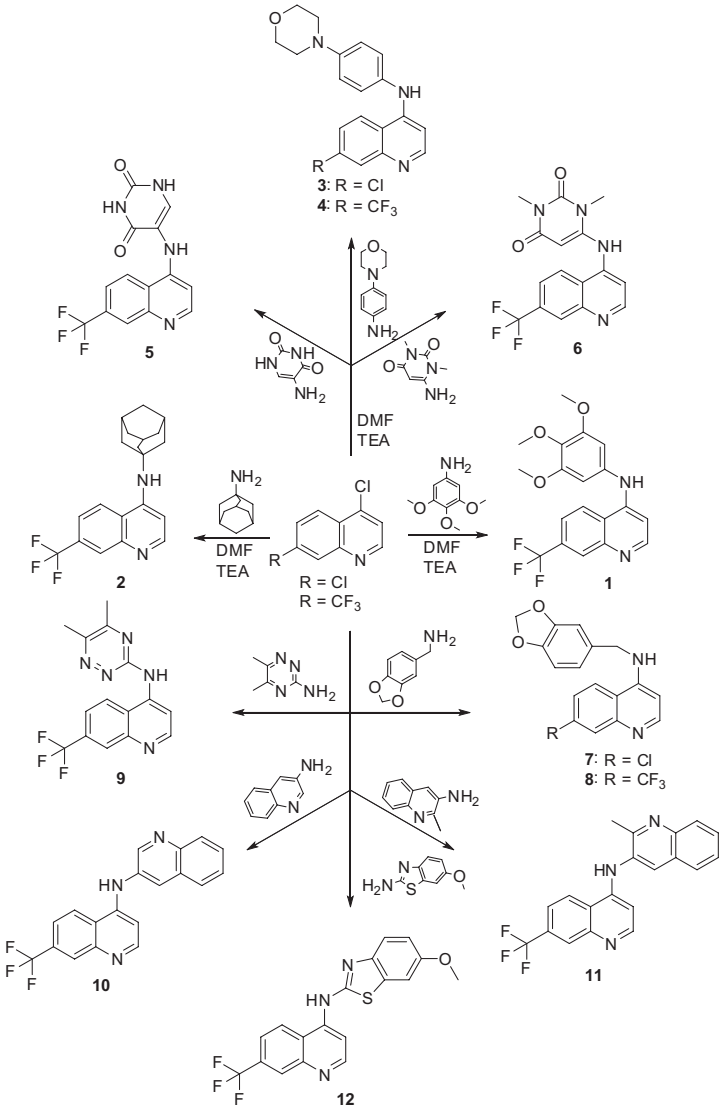
In vitro *anti-proliferative activity*

The cytotoxic activity *in vitro* of newly synthesized compounds was measured using the sulforhodamine B stain (SRB) assay and the method of Skehan (19). The human breast cancer cell lines MCF7 (obtained from National Cancer Institute, Cairo, Egypt) also were maintained at 37 °C in 5 % CO_2 as subconfluent monolayers in 80 cm^3 culture flasks (Nunclon) and were subcultured once or twice weekly in Dulbecco's modification of Eagle's medium (Flow) supplemented with 5 % heat-inactivated fetal calf serum (FCS) and 1 mmol L^{-1} L-glutamine. During experiments, 50 μg mL^{-1} gentamicin was added to the culture medium. Passage levels were in the range of 5–20 according to the original receipt. Cells were harvested from exponential phase cultures by trypsinisation, counted and plated in 96-well flat bottomed microtitre plates (Greiner Labortechnik, Germany) (100 μL cell suspension containing 10^4 cells per well). Following plating and a 24-h recovery to allow cells to resume exponential growth, 100 μL culture medium or culture medium containing the drug was added to the wells. Test compounds were dissolved in DMSO as a 0.1 μmol L^{-1} stock solution (the final concentration of DMSO in culture medium was less than 0.1 %) and diluted with phosphate buffered saline (PBS) to form 10 μmol L^{-1} stock solutions. Different concentrations of each test compound (5, 12.5, 25 and 50 μmol L^{-1}) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in an atmosphere of 5 % CO_2. Forty eight hours after drug addition, cells were fixed with 50 % trichloroacetic acid at 4 °C (50 μL/well) for 1 h, washed with 1 % acetic acid and stained for 30 min with 50 μL of 0.4 % (*m*/*V*) SRB dissolved in 1 % acetic acid. Excess unbound dye was removed by four washes with 1 % acetic acid and attached stain was recovered with Tris-EDTA buffer. Colour intensity was measured using an enzyme-linked immunosorbent assay ELISA reader. Optical density was read at 510 nm. The relation between the surviving fraction and drug concentration was plotted to get the survival curve for the breast cancer cell line (MCF7) after specified time (19). The molar concentration required for 50 % inhibition of cell viability (IC_{50}) was preliminarily calculated from the constructed dose-response curve using Prism software (Graphpad, Inc., USA) and the results are given in Table III.

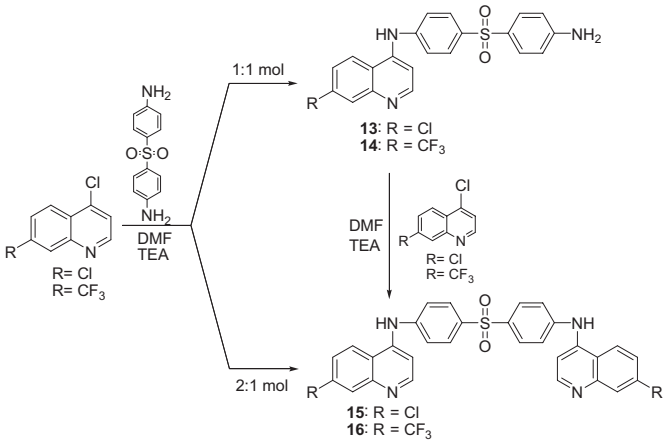
RESULTS AND DISCUSSION

Chemistry

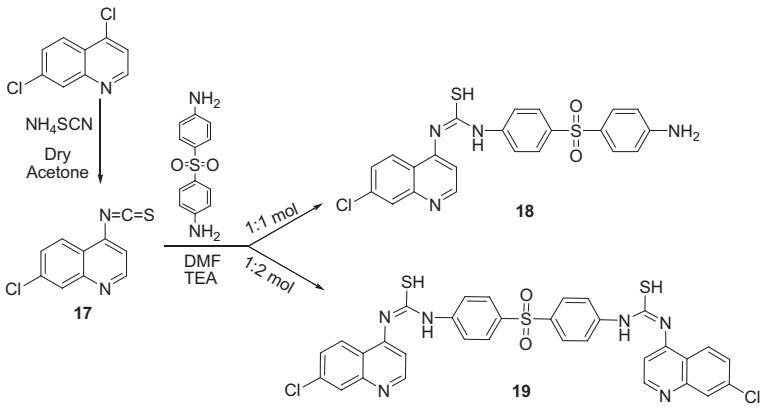
A new series of aminoquinoline derivatives **1–19** was prepared starting from 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline as depicted in Schemes 1-3. The synthesis of quinoline derivatives **1–12** is outlined in Scheme 1. Compounds **1** and **2**



Scheme 1



Scheme 2



Scheme 3

were obtained in good yields *via* the reaction of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline with 3,4,5-trimethoxyaniline and 1-adamantylamine in DMF, respectively. IR spectrum of **1** revealed a characteristic band at 3390 cm^{-1} (NH). ^1H NMR spectrum indicated the presence of signals at 3.8 ppm, which could be assigned to 3 OCH_3 and a singlet at 9.5 ppm for the NH group. ^1H NMR spectrum of compound **2** exhibited signals at 1.64, 1.76 and 2.14 ppm assigned to adamantane protons (Table II). On the other hand, when 4,7-dichloroquinoline and 4-chloro-7-trifluoromethylquinoline reacted with 4-aminomorpholine, the corresponding 4-aminomorpholinophenyl derivatives **3** and **4** were obtained, respectively. IR spectra of compounds **3** and **4** showed the presence of characteristic bands at 3189, 3360 cm^{-1} (NH). ^1H NMR spectra of **3** and **4** indicated the presence of signals at 3.1 and 3.7 ppm for 4 CH_2 of the morpholino moiety. Further, pyrimidine deri-

vatives **5** and **6** were obtained in good yields *via* the reaction of 4-chloro-7-trifluoromethylquinoline with 5-aminouracil and 6-amino-1,3-dimethyl uracil in refluxing DMF, respectively. IR spectra exhibited the presence of an NH and 2 C=O groups. ^{1}H NMR spectrum of **5** revealed a signal at 7.6 ppm assigned to the CH of uracil and others at 11.0 and 11.4 ppm due to the 2 NHs of uracil. ^{1}H NMR of **6** indicated the presence of 2 CH_3 groups at 2.5 and 3.3 ppm and CH uracil at 6.5 ppm (Table II). Interaction of 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline with pipronylamine gave the corresponding benzo[*d*][1,3]dioxol derivatives **7** and **8**, respectively. ^{1}H NMR spectra exhibited signals for CH_2NH at 4.2 and 4.1 ppm and for OCH_2O at 6.0 ppm. The 1,2,4-triazine derivative **9** was obtained through the reaction of 4-chloro-7-trifluoromethylquinoline with 3-amino-5,6-dimethyl-1,2,4-triazine in DMF. ^{1}H NMR spectrum showed signals at 2.3 ppm attributed to the 2 CH_3 groups and 8.8 ppm assigned to the NH group. Also, quinoline derivatives **10** and **11** were obtained *via* the reaction of 4-chloro-7-trifluoromethylquinoline with 3-aminoquinoline and 4-aminoquinaldine, respectively. ^{1}H NMR spectrum of **10** revealed a singlet at 9.0 ppm for the NH group. ^{1}H NMR spectrum of **11** showed a signal at 2.4 ppm for CH_3 and another at 9.2 ppm for the NH group (Table II). Interaction of 4-chloro-7-trifluoromethylquinoline with 6-methoxybenzothiazole afforded the corresponding benzo[*d*] thiazole derivative **12**. ^{1}H NMR spectrum indicated the presence of a triplet at 1.3 ppm for the CH_3 group and a quartet at 4.0 ppm due to the CH_2 group.

On the other hand, and as depicted in Scheme 2, when 4,7-dichloroquinoline or 4-chloro-7-trifluoromethylquinoline reacted with dapsone in a molar ratio 1:1 in DMF, they afforded the corresponding 4-aminophenylsulfonyl derivatives **13** and **14**, respectively, whereas conducting this reaction in a molar ratio 2:1, the corresponding bis-compounds **15** and **16** were obtained in good yields. IR spectra of compounds **13** and **14** showed the presence of free NH_2 bands at 6.2 ppm. ^{1}H NMR spectra of **15** and **16** revealed signals at 11.4 and 9.7 ppm for the 2 NH groups (Table II).

Isothiocyanate derivative **17** was synthesized by treatment of compound 4,7-dichloroquinoline with ammonium thiocyanate in dry acetone for 1 h (Scheme 3). This method led to higher overall yield and shorter working time in comparison with the reported method using silver thiocyanate (19). IR spectrum of **17** showed the characteristic band at 2072 cm^{-1} assigned to N=C=S. Interaction of **17** with dapsone (1:1 mol) yielded the corresponding thiourea derivative **18**, whereas conducting this reaction in molar ratio 2:1 afforded the corresponding bis-compound **19**. Structures of compounds **18** and **19** were supported by elemental analysis and spectral data. IR spectrum of **18** revealed the presence of a band corresponding to free NH_2, while IR spectrum of **19** showed bands at 3440 and 3396 cm^{-1} for 2 NH groups. ^{1}H NMR spectrum of compound **18** exhibited signals at 2.6 ppm assigned to the SH group and 4.9 ppm attributed to the NH_2 group. ^{1}H NMR spectrum of compound **19** revealed signals at 2.7 ppm for the SH group and at 10.7 ppm due to 2 NH groups (Table II).

In vitro *anti-proliferative activity and SAR*

All the newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against the breast cancer cell line (MCF-7) compared with that of doxorubicin. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50 % inhibition of cell viability (Table III). The most active derivative in this study was the 3,4,5-trimethxyphenyl4-aminoquinoline derivative (**1**, IC_{50} = 9.38 μmol L^{-1})

Table II. Spectral data of the newly synthesized compounds

Compd.	IR (ν_{max}, cm^{-1})	^1H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)
1	3390 (NH), 3079 (CH arom.), 2946, 2836 (CH aliph.), 1618 (C=N)	3.8 (s, 9H, $3OCH_3$), 7.0, 8.7 (2d, 2H, 2CH quinoline, J = 7.5 Hz), 7.1, 8.5 (m, 5H, Ar-H), 9.5 (s, 1H, NH, D_2O-exchangeable)	55.7 (2), 56.0, 99.6 (2), 121.0, 122.7, 124.5, 124.6, 127.1, 129.7, 130.4, 133.9, 135.0, 145.6, 149.6, 151.1(2), 153.4
2	3296 (NH), 3079 (CH arom.), 2970, 2862 (CH aliph.), 1621 (C=N)	1.64 (s, 6H, adamantane-H), 1.76 (s, 3H, adamantane-H), 2.14 (s, 6H, adamantane-H), 4.2 (s,1H, NH, D_2O-exchangeable), 7.0, 8.6 (2d, 2H, 2CH quinoline, J = 7.6 Hz), 3.8 (s, 9H, $3OCH_3$), 7.0, 8.7 (2d, 2H, 2CH quinoline, J = 7.5 Hz), 7.6-8.4 (m, 3H, Ar-H), 9.8 (s, 1H, NH, D_2O-exchangeable)	28.9, 35.6 (2), 38.9 (3), 40.3 (3), 52.9, 119.1, 124.4, 124.7, 125.0, 127.1, 129.7, 129.9, 145.5, 148.9, 149.5
3	3189 (NH), 3096 (CH arom.), 2956, 2838 (CH aliph.), 1627 (C=N)	3.1 (s, 4H, N-CH_2-N), 3.7 (s, 4H, O-CH_2-O), 6.8, 8.8 (2d, 2H, 2CH quinoline, J = 7.0 Hz), 7.0-8.4 (m, 7H, Ar-H), 10.7 (s, 1H, NH, D_2O-exchangeable)	48.0 (2), 66.0 (2), 112.3, 115.6 (2), 116.0 (2), 120.5, 125.7, 126.6, 128.2, 133.6, 137.4, 140.7, 144.4, 149.9, 154.1
4	3360 (NH), 3100 (CH arom.), 2973, 2855 (CH aliph.), 1610 (C=N)	3.1, 3.7 (2s, 8H, $4CH_2$), 6.7, 8.6 (2d, 2H, 2CH quinoline, J = 6.9 Hz), 7.0-8.4 (m, 7H, Ar-H), 9.0 (s, 1H, NH, D_2O-exchangeable)	48.0 (2), 66.0 (2), 115.9 (2), 119.3, 120.0 (2), 121.2, 124.1, 125.2, 126.4, 129.1, 129.4, 130.9, 147.8, 148.5, 149.1, 152.2
5	3273, 3196 (NH), 3068 (CH arom.), 1698, 1681 (2C=O), 1623 (C=N)	6.5, 8.5 (2d, 2H, 2CH quinoline, J = 7.8 Hz), 7.6 (s, 1H, CH uracil), 7.7-8.4 (m, 4H, Ar-H), 8.5 (s, 1H, NH, D_2O-exchangeable), 11.0, 11.4 (2s, 2H, 2NH uracil, D_2O-exchangeable)	112.2, 119.0, 120.0, 123.0, 124.0, 125.1, 128.8, 129.3, 129.6, 147.3, 150.3, 151.0, 151.9, 161.8
6	3391 (NH), 3086 (CH arom.), 2961, 2838 (CH aliph.), 1709, 1668 (2C=O), 1628 (C=N)	2.5 (s, 3H, CH_3), 6.5 (s, 1H, CH uracil), 7.5, 8.7 (2d, 2H, 2CH quinoline, J = 8.1 Hz), 7.7-8.6 (m, 3H, Ar-H), 9.1 (s, 1H, NH, D_2O-exchangeable)	28.0, 29.6, 78.0, 114.6, 121.0, 122.8, 123.1, 126.0, 128.8, 136.7, 147.7, 149.4, 151.6, 152.1, 161.8, 166.9
7	3278 (NH), 3068 (CH arom.), 2946, 2873 (CH aliph.), 1612 (C=N), 755 (C-Cl)	4.2 (s, 2H, CH_2), 6.0 (s, 2H, O-CH_2-O), 6.8, 8.8 (2d, 2H, 2CH quinoline, J = 7.4 Hz), 7.0-8.0 (m, 6H, Ar-H), 9.0 (s, 1H, NH, D_2O-exchangeable)	45.7, 103.7, 113.3, 116.7, 119.3, 120.6, 122.1, 125.7, 129.6, 130.6, 137.0 (2), 145.8, 146.9, 149.3, 155.6, 157.3
8	3381 (NH), 3075 (CH arom.), 2951, 2836 (CH aliph.), 1618 (C=N)	4.1 (s, 2H, CH_2), 6.0 (s, 2H, O-CH_2-O), 6.8, 8.7 (2d, 2H, 2CH quinoline, J = 7.1 Hz), 7.0-7.9 (m, 6H, Ar-H), 8.9 (s, 1H, NH, D_2O-exchangeable)	47.8, 102.9, 113.7, 116.9, 117.6, 121.7, 122.9, 124.0, 124.6, 126.7, 130.6, 132.7, 135.0, 144.8, 148.3, 149.1, 150.6, 153.9
9	3412 (NH), 3091 (CH arom.), 2976, 2866 (CH aliph.), 1618 (C=N)	2.3 (s, 6H, $2CH_3$), 6.9, 8.6 (2d, 2H, 2CH quinoline, J = 7.7 Hz), 7.4-8.4 (m, 3H, Ar-H), 8.8 (s, 1H, NH, D_2O-exchangeable)	17.9 (2), 118.4, 124.4, 125.0, 126.7, 127.2, 128.7, 129.2, 140.5, 141.8, 146.2, 148.2, 151.8, 156.4

10	3360 (NH), 3056 (CH arom.), 1621 (C=N)	7.2, 8.7 (2d, 2H, 2CH quinoline, *J* = 7.7 Hz), 7.5-8.4 (m, 9H, Ar-H), 9.0 (s, 1H, NH, D_2O-exchangeable)	115.5, 120.3, 121.6, 122.8, 124.5, 125.4, 126.8, 127.5, 128.6, 129.8 (2), 130.3, 133.7, 142.8, 144.6, 146.6, 147.4, 148.2, 151.4
11	3291 (NH), 3066 (CH arom.), 2976, 2856 (CH aliph.), 1620 (C=N)	2.4 (s, 3H, CH_3), 6.4 (s, 1H, CH quinoline), 6.5, 8.9 (2d, 2H, 2CH quinoline, *J* = 6.9 Hz), 7.2-8.3 (m, 7H, Ar-H), 9.2 (s, 1H, NH, D_2O-exchangeable)	24.7, 110.9, 117.2, 120.3, 122.5, 122.9, 123.1, 124.6, 126.8, 127.3, 128.0, 130.1, 130.6, 130.9, 147.3, 148.4, 151.4, 152.2, 153.5, 158.1
12	3312 (NH), 3086 (CH arom.), 2961, 2846 (CH aliph.), 1612 (C=N)	1.3 (t, 3H, CH_3), 4.0 (q, 2H, CH_2), 6.7, 8.8 (2d, 2H, 2CH quinoline, *J* = 7.0 Hz), 6.9-8.4 (m, 6H, Ar-H), 10.8 (s, 1H, NH, D_2O-exchangeable)	14.6, 63.5, 105.5, 115.3, 118.0, 120.7, 121.3, 122.8, 123.9, 125.0, 126.7, 131.8, 132.8, 142.3, 148.2, 151.9, 153.4, 155.4, 164.6
13	3361, 3348, 3215 (NH, NH_2), 3100 (CH arom.), 1593 (C=N), 1375, 1147 (SO_2), 748 (C-Cl)	6.2 (s, 2H, NH_2, D_2O-exchangeable), 6.7, 8.8 (2d, 2H, 2CH quinoline, *J* = 7.4 Hz), 7.1-8.3 (m, 11H, Ar-H), 10.8 (s, 1H, NH, D_2O-exchangeable)	112.9, 119.3 (4), 121.7, 123.3, 127.2, 129.1 (4), 129.3, 135.3 (2), 137.4, 146.3, 148.7, 149.4, 151.7, 153.4
14	3417, 3365, 3310 (NH, NH_2), 3091 (CH arom.), 1594 (C=N), 1381, 1145 (SO_2)	6.2 (s, 2H, NH_2, D_2O-exchangeable), 6.6, 8.8 (2d, 2H, 2CH quinoline, *J* = 6.8 Hz), 7.3-8.3 (m, 11H, Ar-H), 10.7 (s, 1H, NH, D_2O-exchangeable)	117.0, 119.3 (4), 121.9, 123.3, 124.5, 125.8, 127.8, 128.9 (2), 129.2 (2), 130.1, 130.5 (2), 145.6, 147.8, 152.7, 153.5, 160.2
15	3421, 3267 (NH), 3079 (CH arom.), 1616 (C=N), 1361, 1151 (SO_2), 788 (C-Cl)	7.1, 8.6 (2d, 2H, 2CH quinoline, *J* = 7.9 Hz), 7.5-8.2 (m, 14H, Ar-H), 11.4 (s, 2H, 2NH, D_2O-exchangeable)	116.6 (2), 119.6 (4), 124.0 (2), 124.5 (2), 126.4 (2), 127.6 (4), 129.2 (2), 138.4 (2), 139.5 (2), 142.5 (2), 144.2 (2), 153.7 (2), 162.2 (2)
16	3381, 3267 (NH), 3055(CH arom.), 1619 (C=N), 1372, 1162 (SO_2)	6.6, 8.6 (2d, 2H, 2CH quinoline, *J* = 7.5 Hz), 7.3-8.5 (m, 14H, Ar-H), 9.7 (s, 2H, 2NH, D_2O-exchangeable)	112.9 (2), 119.5 (4), 122.7 (2), 123.0 (2), 124.7 (2), 126.3 (2), 128.9 (2), 129.9 (4), 134.4 (4), 146.3 (2), 147.7 (2), 149.6 (2), 152.2 (2)
17	2072 (N=C=S) 1616 (C=N), 879 (C-Cl)	6.9, 8.9 (2d, 2H, 2CH quinoline, *J* = 6.8 Hz), 7.2-8.4 (m, 3H, Ar-H)	118.8, 126.6, 127.7, 129.8, 130.6, 136.4, 138.9, 140.4, 150.9, 152.0
18	3446, 3386, 3290 (NH, NH_2), 3081 (CH arom.), 1595 (C=N), 1381, 1182 (SO_2), 833 (C-Cl)	2.6 (s, 1H, SH, D_2O-exchangeable), 4.9 (br s, 2H, NH_2, D_2O-exchangeable), 6.6, 8.6 (2d, 2H, 2CH quinoline, *J* = 6.9 Hz), 7.0-8.4 (m, 7H, Ar-H), 10.8 (s, 1H, NH, D_2O-exchangeable)	113.0, 119.2 (4), 125.0, 128.5, 129.1 (5), 129.5, 137.4 (2), 139.0, 147.8, 148.1, 152.5, 153.6, 160.2
19	3446, 3396 (NH), 1596 (C=N), 1319, 1145 (SO_2)	2.7 (s, 2H, 2SH, D_2O-exchangeable), 6.6, 8.9 (2d, 4H, 4CH quinoline, *J* = 7.2 Hz), 7.4-8.3 (m, 14H, Ar-H), 10.7 (s, 2H, 2NH, D_2O-exchangeable)	115.8 (2), 119.1 (4), 124.8 (2), 127.8 (2), 128.2 (6), 128.4 (2), 129.1 (2), 142.1 (2), 144.7 (2), 153.4 (2), 155.9 (2), 160.3 (2), 162.3 (2)

Table III. In vitro *anti-proliferative screening of the newly synthesized compounds against the human breast cancer cell line (MCF-7)*

Compd.	Compound concentration (μmol L^{-1})				IC_{50} (μmol L^{-1})
	5	12.5	25	50	
	Surviving fraction[a]				
Doxo-rubicin	0.551 ± 0.026	0.480 ± 0.003	0.139 ± 0.005	0.130 ± 0.016	32.00
1	0.512 ± 0.017	0.488 ± 0.015	0.442 ± 0.003	0.400 ± 0.027	9.38
2	0.801 ± 0.012	0.650 ± 0.041	0.485 ± 0.047	0.453 ± 0.022	24.10
3	0.873 ± 0.074	0.562 ± 0.033	0.523 ± 0.004	0.442 ± 0.021	31.50
4	0.746 ± 0.032	0.625 ± 0.024	0.481 ± 0.023	0.391 ± 0.015	23.30
5	0.835 ± 0.021	0.638 ± 0.058	0.442 ± 0.004	0.356 ± 0.016	21.40
6	0.775 ± 0.044	0.580 ± 0.012	0.489 ± 0.014	0.458 ± 0.065	23.30
7	0.692 ± 0.002	0.606 ± 0.009	0.450 ± 0.028	0.310 ± 0.047	21.10
8	0.895 ± 0.085	0.581 ± 0.016	0.508 ± 0.022	0.335 ± 0.043	26.20
9	0.861 ± 0.020	0.642 ± 0.020	0.452 ± 0.010	0.394 ± 0.030	21.80
10	0.686 ± 0.064	0.520 ± 0.014	0.358 ± 0.016	0.302 ± 0.012	14.20
11	0.630 ± 0.064	0.562 ± 0.014	0.345 ± 0.016	0.357 ± 0.012	16.30
12	0.777 ± 0.002	0.620 ± 0.009	0.591 ± 0.028	0.600 ± 0.047	NA[b]
13	0.764 ± 0.058	0.569 ± 0.026	0.436 ± 0.030	0.345 ± 0.022	18.80
14	0.669 ± 0.002	0.596 ± 0.009	0.487 ± 0.028	0.392 ± 0.047	23.50
15	0.857 ± 0.002	0.679 ± 0.009	0.474 ± 0.028	0.440 ± 0.047	23.20
16	0.827 ± 0.058	0.650 ± 0.026	0.487 ± 0.030	0.402 ± 0.240	24.00
17	0.853 ± 0.002	0.713 ± 0.009	0.447 ± 0.028	0.415 ± 0.047	22.40
18	0.895 ± 0.017	0.748 ± 0.015	0.444 ± 0.003	0.397 ± 0.027	22.70
19	0.564 ± 0.011	0.440 ± 0.010	0.142 ± 0.007	0.149 ± 0.013	33.60

[a] Mean ± SD, $n = 3$.

[b] NA – no activity observed under the adopted experimental conditions.

showing excellent anti-proliferative efficacy, being almost thrice as potent as doxorubicin (IC_{50} = 32.00 μmol L^{-1}). Also, 3-quinolinyl derivatives (**10**, IC_{50} = 14.20 μmol L^{-1}) and its 2-methyl analogue (**11**, IC_{50} = 16.30 μmol L^{-1}) displayed very high activity, being approximately double as potent as doxorubicin.

To investigate the effect of increasing lipophilicity on the biological activity of this class of compounds, the pyrimidine-2,4-dione derivative **5** and its 1,3-dimethyl analogue **6** were prepared. Cytotoxic screening results showed that the more lipophilic derivative

6 with IC_{50} = 23.30 μmol L^{-1} was slightly less active than its dimethyl counterpart **5** with IC_{50} = 21.40 μmol L^{-1}. Similar findings were observed for the quinoline-4-yl derivative **10** and its 2-methylquinolin-4-yl isostere **11** displaying IC_{50} values of 14.20 and 16.30 μmol L^{-1}, respectively.

Regarding bis-compounds, there seemed to be no priority for preparing the bis-analogues over their counterparts, since they did not demonstrate any enhancement of activity. Looking at the data for compounds **13** and **14** (IC_{50} values 18.80 and 23.50 μmol L^{-1}, respectively) and their respective bis-compound derivatives **15** and **16** (IC_{50} values 23.20 and 24.00 μmol L^{-1}, respectively), a reduction in activity was observed. A more explicit example was evidenced by compound **18** and its bis-compound **19** eliciting IC_{50} values of 22.70 and 33.60 μmol L^{-1}, respectively.

While seventeen out of nineteen synthesized compounds showed higher potency than doxorubicin, dapsone bis-compound (**19**) was almost as active as doxorubicin (IC_{50} of 33.60 *viz.* 32.00 μmol L^{-1}) and benzothiazole derivative (**12**) was not active in the adopted experimental procedure.

Finally, it is noteworthy that the cells killing potency of the 7-chloro derivatives was usually better than that of their 7-trifluoromethyl. Thus, compound **7** having a chloro group at 7-position with IC_{50} value 21.10 μmol L^{-1} was more active than compound **8** bearing trifluoromethyl moiety at the same position IC_{50} value 26.20 μmol L^{-1}. Also, compound **13** with IC_{50} of 18.80 μmol L^{-1} showed a significant cytotoxic activity, which was even higher than countercompound **14** with IC_{50} 23.50 μmol L^{-1}. In addition, bis-compound **15** carrying a chloro group (IC_{50} value 23.20 μmol L^{-1}) was found to possess higher potency than bis-compound **16** incorporating trifluoromethyl moiety IC_{50} value 24.00 μmol L^{-1}. The presence of the chloro group at position 7 in compounds **7**, **13**, **15** is supposed to enhance their anticancer activity. An exception was observed by the analogous pair **3** and **4** where 7-chloro derivative **3** had lower potency (IC_{50} 31.50 and 23.30 μM, respectively). It is clear from the present data that the comparison of the cytotoxicity of quinoline derivatives against the breast cancer cell line (MCF7) (Table III) has shown that the cell killing potency follows the order **1** > **10** > **11** > **13** > **7** > **5** > **9** > **17** > **18** > **15** > **4** > **6** > **14** > **16** > **2** > **8** > **3** > doxorubicin.

CONCLUSIONS

The objective of the present study was to synthesize and investigate the anti-proliferative activity of some novel derivatives based on a 4-aminoquinoline backbone carrying variable biologically active moieties, *viz*., trimethoxyphenyl, adamantyl, pyrimidin-2,4-dione, benzodioxole and dapsone, as well as a few bis-compounds. The anti-proliferative screening results of the new compounds against MCF-7 showed that seventeen compounds were more potent than the reference drug doxorubicin. Among them, 7-(trifluoromethyl)-*N*-(3,4,5-trimethoxyphenyl)quinolin-4-amine (**1**), *N*-(7-(trifluoromethyl)quinolin-4-yl)quinolin-3-amine (**10**), 2-methyl-*N*-(7-trifluoromethyl)quinolin-4-yl)quinolin-3-amine (**11**) and *N*-(4-(4-aminophenylsulfonyl)phenyl)-7-chloroquinolin-4-amine (**13**). **1**, **10** and **11** were almost twice to thrice as potent as doxorubicin. We have inferred from the biological assessment data that this class of derivatives represents a very promising starting point for the design and synthesis of more analogues with further improved anti-proliferative activity. *In vivo* and toxicity studies of these compounds are under way.

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