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Synthesis, DNA binding and cytotoxic evaluation of aminoquinoline scaffolds

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Abstract. An effortless synthetic route has been developed for the synthesis of a new class of aminoquinoline substituted isoindolin-1,3-diones from regio-isomerical hydrazinylquinolines with phthalic anhydride in presence of Eaton's reagent. DNA binding studies of selected isomeric compounds showed interaction with DNA *via* intercalation mode with higher binding affinity of 4-substituted quinolines rather than 2-substituted counterparts. Further, all compounds were screened for cytotoxic activity against three human cancer cell lines, among them compound **2c** outranged standard doxorubicin against CCRF-CEM cell line.

Keywords. Hydrazinylquinolines; Isoindolin-1,3-diones; DNA binding; cytotoxicity.

1. Introduction

Nitrogen containing heterocyclic compounds are renowned as significant biologically active agents in medicinal chemistry.[1] Aminoquinolines have emerged as one of the major therapeutic agents in malarial treatment, such as chloroquine and amodiaquine,[2] which were frontline antimalarial drugs used for several decades. The well-known aminoquinoline scaffold, chloroquine, has recently been in focus due to its potential biological effects on cancer cells, such as the inhibition of cell growth and/or induction of cell death in human lung cancer A549 cells, glioma cells, human breast cancer cells, and mouse colon cancer CT29 cells resulting in anticancer effects.[3] Nevertheless, empirical screening continues to be essential for discovering such aminoquinoline derived compounds with potent anticancer activity.[4] Cyclic imides are another important class of compounds because of their wide range of interesting biological properties.[5] Among the existing various N-substituted cyclic imides, isoindolin-1,3-dione derivatives have been identified as one of the most promising scaffold, which contain the general structure –CO-N(R)-CO-, so that they are hydrophobic and neutral, and can therefore cross biological membranes *in vivo*.[6] As shown in figure 1, by introduction of nitrogen-containing heterocycle into the isoindolin-1,3-diones (**1, 2**), they are found to exhibit potent antibacterial and antifungal activities[6,7] and as potential antitumor agent inhibiting cyclin-dependent kinases (CDKs).[8]

DNA is a remarkable bioreceptor for a vast number of small molecules, and it remains a major biological target for the design of anticancer agents. It is generally believed that the molecules bind with DNA, and obstruct DNA synthesis indirectly, leading to disruption of hormonal regulation of cell growth which make them better suited for developing chemotherapeutic agents.[9] Therefore, the investigation of small molecules towards DNA binding properties makes pathway for identifying new anticancer agents. The capability of quinoline derivatives for DNA binding activity have been extensively studied.[10] Quinolines can intercalate DNA duplex, for example, amidino-substituted benzo[*b*]thieno [2,3-*c*]quinolones and benzothiazoloquinoline[11] have been studied for their DNA binding properties. Different evidences have demonstrated the ability of quinolines as potential antitumor agents.[12] The antitumor potential of quinolines against human breast cancer cells (MCF7), in particular, chloroquine as the most potent apoptosis inducing agent, has been reported.[13] Based on the above significant biological activities

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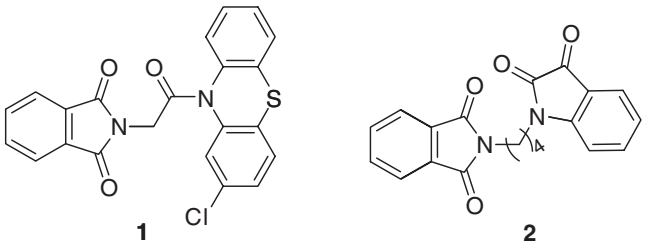


Figure 1. Bioactive isoindolin-1,3-dione derivatives.

associated with the core units of quinolines and isoindolin-1,3-diones, we have been interested to develop a new synthetic route for the synthesis of quinoline substituted isoindolin-1,3-diones. Further, we have investigated selective compounds, i.e., compounds with more aromatic rings for enhanced binding ability with calf thymus DNA (CT-DNA) due to increased binding affinity of aromatic residues with DNA base pairs[14] and in competitive binding studies with known intercalating agent ethidium bromide (EB). All the synthesized compounds were screened for *in vitro* cytotoxicity against three human cancer cell lines and a few of them exhibited significant cytotoxic activity towards the tested cancer cell lines.

2. Experimental

2.1 *General information*

Melting points (M.p.) were determined on a Mettler FP 51 apparatus (Mettler Instruments, Switzerland) and are uncorrected. They are expressed in degree centigrade (°C). A Nicolet Avatar Model FT-IR spectrophotometer was used to record the IR spectra (4000–400 cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AV 500 (500 MHz (^1H) and 125 MHz (^{13}C)) and Bruker AV 400 (400 MHz (^1H) and 100 MHz (^{13}C)) spectrometers using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Coupling constants (J) are reported in hertz (Hz). The terms J_o and J_m refer to ortho and meta coupling constants. The terms **s**, **d**, **t**, **dd** refer to singlet, doublet, triplet and doublet of doublet, respectively, **b s** refers to a broad singlet. Mass spectra (MS), were recorded on an Auto Spec EI+ Shimadzu QP 2010 PLUS GC-MS and Perkin Elmer Clarus 600 (EI) mass spectrometer. Microanalyses were performed on a Vario EL III model CHNS analyzer (Vario, Germany) at the Department of Chemistry, Bharathiar University. Eaton's reagent was purchased from Sigma Aldrich. Unless otherwise specified, other reagents were obtained from commercial suppliers. When known compounds had to be prepared according to literature procedures, pertinent references are given. The purity of the products was tested by TLC with plates coated with silica gel-G using petroleum ether and ethyl acetate in the ratio of 50:50 as developing solvents. Columns packed with activated silica gel (60-120 mesh) were used to purify the product.

2.2 *General procedure for the preparation of aminoquinoline substituted isoindolin-1,3-diones **3(a-d)** and **4 (a-d)***

The respective hydrazinylquinoline (**1**(**a**-**d**)/**2**(**a**-**d**), 0.001 mol) was heated with phthalic anhydride (0.002 mol, 0.296 g) in presence of Eaton's reagent (1 mL) at 90°C in heating mantle for 3 h. Then, the reaction mass was poured to an excess of saturated sodium carbonate solution (30 mL). The solid obtained was filtered, and washed with sufficient water and dried. The crude product was recrystallized from ethanol to afford aminoquinoline substituted isoindolin-1,3-diones (**3**(**a**-**d**) and **4**(**a**-**d**)) in good yield.

2.2a *2-(2′-Chloro-benzo[h]quinolin-4′-ylamino)-isodolin-1,3-dione (**3a**)*: White solid; Yield: 91%; M.p. >300°C; IR (KBr, cm^{-1}): 3347, 1724; ^1H NMR ($CDCl_3$, 500 MHz): δ 6.61 (s, 1H, C_3'-H), 7.40 (s, 1H, N-H), 7.67-7.73 (m, 3H, C_6', C_8', C_9'-H), 7.81 (d, 1H, C_5'-H, J = 9.0 Hz), 7.90 (d, 1H, C_7'-H, J = 7.5 Hz), 7.91-8.05 (m, 4H, C_4, C_5, C_6, C_7-H), 9.08 (dd, 1H, C_{10}'-H, J_o = 7.5 Hz, J_m = 1.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 102.60, 113.48, 116.33, 124.43, 125.26, 127.36, 127.43, 127.64, 128.76, 129.64, 130.55, 133.65, 135.26, 146.71, 150.01, 150.47, 165.48; GC-MS (EI): m/z (%) 373.07 (100) [M+]; Anal. Calcd. for: $C_{21}H_{12}ClN_3O_2$: C, 67.48; H, 3.24; N, 11.24%. Found: C, 67.43; H, 3.25; N, 11.27%.

2.2b *2-(2′-Chloro-6′-methyl-quinolin-4′-ylamino)-isoindolin-1,3-dione (**3b**)*: White solid; Yield: 89%; M.p. >300′C; IR (KBr, cm^{-1}): 3348, 1721; ^1H NMR (DMSO-d_6, 500 MHz): δ 2.54 (s, 3H, C_6'-CH_3), 6.89 (s, 1H, C_3'-H), 7.65 (dd, 1H, C_7'-H, J_o = 9.0 Hz, J_m = 1.5 Hz), 7.76 (d, 1H, C_8'-H, J = 8.5 Hz), 7.96-8.03 (m, 4H, C_4, C_5, C_6, C_7-H), 8.06 (s, 1H, C_5'-H), 10.01 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 21.79, 100.25, 116.68, 121.19, 124.23, 128.58, 130.36, 133.19, 135.56, 135.97, 146.36, 150.65, 151.43, 166.15; GC-MS (EI): m/z (%) 337.10 (100) [M+]; Anal. Calcd. for: $C_{18}H_{12}ClN_3O_2$: C, 64.01; H, 3.58; N, 12.44%. Found: C, 64.07; H, 3.56; N, 12.41%.

2.2c *2-(2′-Chloro-8′-methyl-quinolin-4′-ylamino)-isoindolin-1,3-dione (**3c**)*: White solid; Yield: 90%; M.p. 278-280°C; IR (KBr, cm^{-1}): 3363, 1721; ^1H NMR ($CDCl_3$, 500 MHz): δ 2.24 (s, 3H, C'_8-CH_3), 7.05 (s, 2H, C'_3-H & N-H), 7.31 (d, 1H, C'_7-H, J = 8.0 Hz), 7.43 (t, 1H, C'_6-H, J = 7.0 Hz), 7.86-8.01 (m, 5H, C_4, C_5, C'_5, C_6, C_7-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ17.47, 108.97, 121.92, 123.29, 123.85, 124.58, 130.38, 131.03, 134.75, 135.47, 144.48, 146.29, 152.41, 166.86; GC-MS (EI): m/z (%) 337.17 (100) [M+]; Anal. Calcd. for: $C_{18}H_{12}ClN_3O_2$: C, 64.01; H, 3.58; N, 12.44%. Found: C, 64.04; H, 3.57; N, 12.42%.

2.2d *2-(2′,6′-Dichloro-quinolin-4′-ylamino)-isoindolin-1,3-dione (**3d**)*: White solid; Yield: 89%; M.p. >300°C; IR (KBr, cm^{-1}): 3362, 1723; ^1H NMR (DMSO-d_6, 500 MHz): δ 7.07 (s, 1H, C'_3-H), 7.84 (dd, 1H, C'_7-H, J_o = 9.0 Hz, J_m = 2.0 Hz), 7.89 (d, 1H, C_8-H, J = 9.0 Hz), 7.96-8.03 (m, 4H, C_4, C_5, C_6, C_7-H), 8.42 (d, 1H, C'_5-H, J = 2.5 Hz), 10.22 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 101.21, 117.56, 121.58, 124.25, 130.39, 130.95, 131.77, 135.49, 135.54, 146.44, 151.39, 152.12, 165.98; GC-MS (EI): m/z (%) 357.05 (100) [M+]; Anal. Calcd. for: $C_{17}H_9Cl_2N_3O_2$: C, 57.01; H, 2.53; N, 11.73%. Found: C, 57.06; H, 2.51; N, 11.71%.

2.2e *2-(4′-Chloro-benzo[h]quinolin-2′-ylamino)-isoindolin-1,3-dione (**4a**)*: White solid; Yield: 89%; M.p. 178-180°C; IR (KBr, cm^{-1}): 3347, 1721; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.09 (s, 1H, C'_3-H), 7.14 (s, 1H, N-H), 7.43 (t, 1H, C'_9-H, J = 7.5 Hz), 7.60 (t, 1H, C'_8-H, J = 7.5 Hz), 7.72 (d, 1H, C'_6-H, J = 9.0 Hz), 7.84 (d, 1H, C'_5-H, J = 9.0 Hz), 7.91-7.93 (m, 2H, C_5, C_6-H), 7.99 (d, 1H, C'_7-H, J = 9.0 Hz), 8.03-8.06 (m, 2H, C_4, C_7-H), 8.62 (dd, 1H, C'_{10}-H, J_o = 8.0 Hz, J_m = 1.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 108.37, 120.57, 120.81, 124.11, 124.72, 125.97, 126.59, 127.61, 128.44, 130.16, 130.27, 134.16, 134.88, 144.29, 146.14, 153.82, 166.60; GC-MS (EI): m/z (%) 373.09 (100) [M+]; Anal. Calcd. for: $C_{21}H_{12}ClN_3O_2$: C, 67.48; H, 3.24; N, 11.24%. Found: C, 67.46; H, 3.25; N, 11.23%.

2.2f *2-(4′-Chloro-6′-methyl-quinolin-2′-ylamino)-isoindolin-1,3-dione (**4b**)*: White solid; Yield: 87%; M.p. >300°C ; IR (KBr, cm^{-1}): 3347, 1722; ^1H NMR (DMSO-d_6, 500 MHz): δ 2.54 (s, 3H, C'_6-CH_3), 6.89 (s, 1H, C'_3-H), 7.65 (dd, 1H, C'_7-H, J_o = 9.0 Hz, J_m = 1.5 Hz), 7.77 (d, 1H, C'_8-H, J = 8.5 Hz), 7.96-8.03 (m, 4H, C_4, C_5, C_6, C_7-H), 8.06 (s, 1H, C'_5-H), 10.02 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 21.78, 100.25, 116.68, 121.19, 124.22, 128.57, 130.35, 133.32, 135.54, 135.97, 146.36, 150.64, 151.43, 169.12; GC-MS (EI): m/z (%) 337.14 (100) [M+]; Anal. Calcd. for: $C_{18}H_{12}ClN_3O_2$: C, 64.01; H, 3.58; N, 12.44%. Found: C, 64.07; H, 3.56; N, 12.42%.

2.2g *2-(4′-Chloro-8′-methyl-quinolin-2′-ylamino)-isoindolin-1,3-dione (**4c**)*: White solid; Yield: 88%; M.p. 269-271°C; IR (KBr, cm^{-1}): 3348, 1724; ^1H NMR ($CDCl_3$, 500 MHz): δ 2.72 (s, 3H, C'_8-CH_3), 6.42 (s, 1H, C'_3-H), 7.35 (t, 1H, C'_6-H, J = 7.5 Hz), 7.44 (s, 1H, N-H), 7.55 (d, 1H, C'_7-H, J = 7.0 Hz), 7.66 (d, 1H, C'_5-H, J = 8.5 Hz), 7.90-8.03 (m, 4H, C_4, C_5, C_6, C_7-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 18.44, 101.07, 116.57, 117.35, 124.39, 125.78, 129.64, 130.98, 135.22, 137.35, 147.08, 150.02, 150.06, 165.47; GC-MS (EI): m/z (%) 337.12 (100) [M+]; Anal. Calcd. for: $C_{18}H_{12}ClN_3O_2$: C, 64.01; H, 3.58; N, 12.44%. Found: C, 64.03; H, 3.58; N, 12.42%.

2.2h *2-(4′,6′-Dichloro-quinolin-2′-ylamino)-isoindolin-1,3-dione (**4d**)*: White solid; Yield: 87%; M.p. >300°C; IR (KBr, cm^{-1}): 3347, 1721; ^1H NMR (DMSO-d_6, 400 MHz): δ 7.01 (s, 1H, C'_3-H), 7.77 (dd, 1H, C'_7-H, J_o = 8.8 Hz, J_m = 2.0 Hz), 7.82 (d, 1H, C'_8-H, J = 9.2 Hz), 7.88-7.96 (m, 4H, C_4, C_5, C_6, C_7-H), 8.34 (d, 1H, C'_5-H, J = 2.0 Hz), 10.16 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 100.72, 117.05, 121.09, 123.77, 129.90, 130.44, 131.29, 135.06, 135.48, 145.94, 150.91, 151.64, 165.51; GC-MS (EI): m/z (%) 357.03 (100) [M+]; Anal. Calcd. for: $C_{17}H_9Cl_2N_3O_2$: C, 57.01; H, 2.53; N, 11.73%. Found: C, 56.97; H, 2.54; N, 11.75%.

2.3 *DNA binding studies*

All the experiments involving the binding of compounds with CT-DNA were carried out in double distilled water with tris (hydroxymethyl)-aminomethane (Tris, 5 mmol L^{-1}) and sodium chloride (50 mmol L^{-1}) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 L mol^{-1} cm^{-1} at 260 nm. The compounds were dissolved in a mixed solvent of 5% DMSO and 95% Tris-HCl buffer for all the experiments. Absorption titration experiments were performed with a fixed

concentration of the compounds (25 mmol L^{-1}) while gradually increasing the concentration of DNA (5-25 mmol L^{-1}). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself. The same experimental procedure was followed for emission studies also. Further support for the complexes binding to DNA *via* intercalation was given through emission quenching experiments. DNA was pretreated with ethidium bromide for 30 min. Then the test solutions were added to this mixture of EB-DNA, and the change in the fluorescence intensity was measured. The excitation and emission wavelengths were 515 nm and 603-607 nm, respectively.

2.4 *In vitro cytotoxicity*

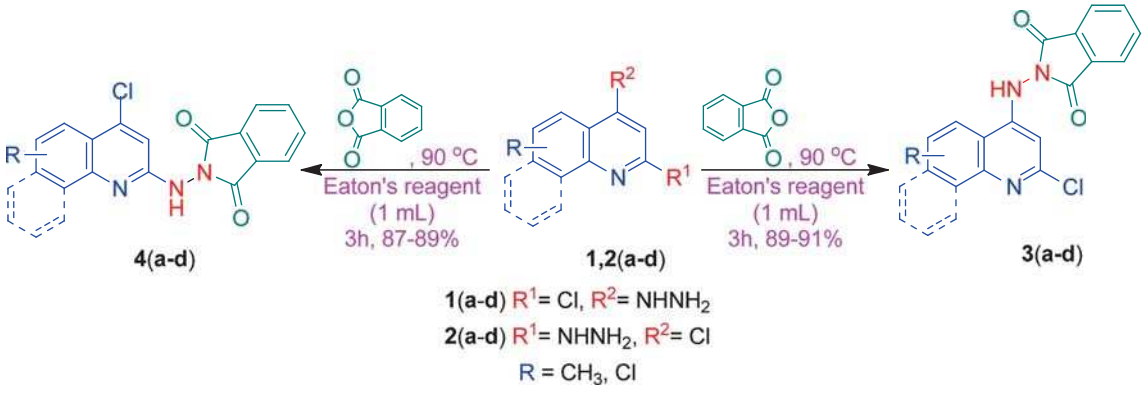
All the tested cell lines were obtained from American Type Culture Collection. The cells were grown on 75 cm^2 cell culture flasks with RPMI-16 medium for CCRF-CEM cells and EMEM (Eagle's minimum essential medium) for HT29 and MCF7 cells, and supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO2, 95% air at 37°C. The cell proliferation assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75-80% confluency, HT29 (5000 cells per well), MCF7 (5000 cells per well), or CCRF-CEM (40,000 cells per well) were plated in 96- well microplate in 100 μL media. After seeding for 72 h, the cells were treated with 50 μmol L^{-1} compound in triplicate. Doxorubicin (10 μmol L^{-1}) was used as the positive control. At the end of the sample exposure period (72 h), 20 μL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37°C. The absorbance of the formazan product was measured at 490 nm using a microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%). The percentage of cell survival was calculated as (OD value of cells treated with the test compound - OD value of culture medium)/(OD value of control cells - OD value of culture medium) × 100%. The percentage inhibition was used to determine the IC50 values. The results are given as mean ± standard deviation.

3. Results and Discussion

3.1 *Chemistry*

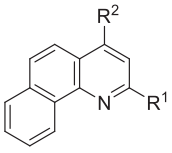
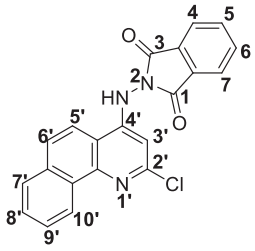
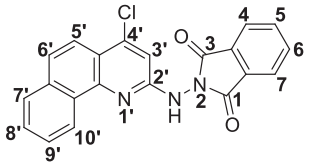
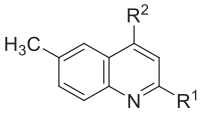
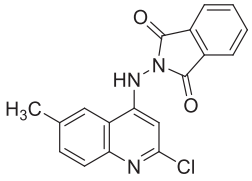
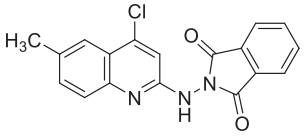
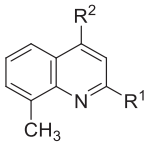
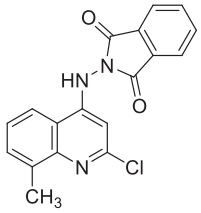
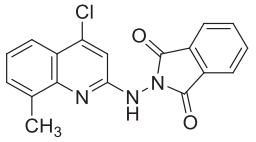
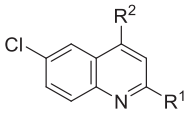
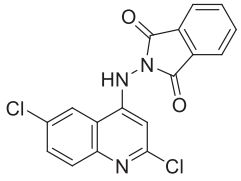
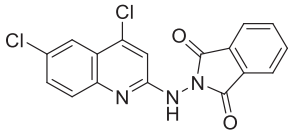
A new class of aminoquinoline substituted isoindolin-1,3-diones were synthesized from regioisomers of hydrazinylquinolines with phthalic anhydride in presence of Eaton's reagent (7.7 wt% phosphorus pentoxide solution in methanesulfonic acid) at 90°C based on our previous reported work,[15] shown in scheme 1.

The IR spectrum of the compound **3a** showed a strong stretching at 3348 cm^{-1} due to the presence of N-H group and a stretching at 1721 cm^{-1} due to C=O group. Its ^{1}H NMR spectrum exhibited two singlets at δ 6.61 and 7.40 due to C_3'-H and N-H protons, respectively. The aromatic protons appeared in the region of δ 7.67-9.08. All the spectral and analytical data revealed the product as 2-(2′-chloro-benzo[*h*]quinolin-4′-yl)amino-isoindolin-1,3-dione (**3a**). The generality of the same reaction was performed on other 2-chloro-4-hydrazinylquinoline (**1b**-**1d**) and 4-chloro-2-hydrazinylquinoline (**2a**-**2d**) with phthalic anhydride to yield the corresponding quinoline substituted isoindolin-1,3-diones (**3b**-**3d**, **4a**-**4d**), table 1.



Scheme 1. Synthesis of aminoquinoline substituted isoindolin-1,3-diones.

Table 1. Synthesis of aminoquinoline substituted isoindolin-1,3-diones (**3**(**a**-**d**) and **4**(**a**-**d**)).

Substrates **1/2**(**a**-**d**)[a]	Product **3**(**a**-**d**) from **1**(**a**-**d**)	Product **4**(**a**-**d**) from **2**(**a**-**d**)
		
		
		
		

[a]Substrate **1a**-**1d**, $R^1 = Cl$, $R^2 = NHNH_2$, Substrate **2a**-**2d**, $R^1 = NHNH_2$, $R^2 = Cl$.

3.2 *DNA binding studies*

DNA binding studies are a commonly used preliminary testing method for antitumor activity of compounds. It is important to know the type of interaction between small molecules with double helix demonstrate the underlying mechanism of binding mode. In general, the molecules bind with DNA through three different binding modes, electrostatic, groove and intercalative. Most of the heteroaromatic molecules exhibit intercalative mode of binding with DNA because of the larger planarity of the compound with extended conjugation and the greater stacking interaction between the compound and base pairs of DNA.[16] In order to describe the mode and efficiency of our newly synthesized compounds with calf-thymus DNA (CT-DNA), different techniques have been used to study the interaction such as absorption titration, fluorescence titration, and competitive ethidium bromide (EB) displacement assay. Compounds binding with DNA through intercalation usually cause hypochromism with or without a small red or blue shift in UV-Visible absorption spectra because the intercalation mode involves a strong π-π* stacking interaction between an aromatic chromophore and the base pairs of DNA.[17]

The absorption spectra of tested compounds **1a**-**4a** (figure 2), in the absence and presence of CT-DNA have shown that increase in the concentration of CT-DNA to tested compounds causes hypochromism of various percentage (20-52% with 1-3 nm). Among the tested compounds, **3a** showed larger hypochromism than other compounds which revealed that it bound more strongly with CT-DNA. In order to compare the binding strength of the compounds, their intrinsic binding constants (K_b) were calculated from the ratio of the slope to intercept in the plot of [DNA]/(ε_a-ε_f) versus [DNA] (figure 3), as explained below.

The intrinsic binding constants (Kb) of the compounds with CT-DNA were determined from the following equation: [DNA]/($\varepsilon_a - \varepsilon_f$) = [DNA]/($\varepsilon_a - \varepsilon_f$) + 1/Kb ($\varepsilon_b - \varepsilon_f$), where [DNA] is the concentration of DNA in base pairs, and the apparent absorption coefficient ε_a corresponds to Aobs/[compound], and ε_f, and ε_b are the extinction coefficient of the free compound, and the extinction coefficient of the compound when fully bound to DNA, respectively. The plot of [DNA]/($\varepsilon_a - \varepsilon_f$)

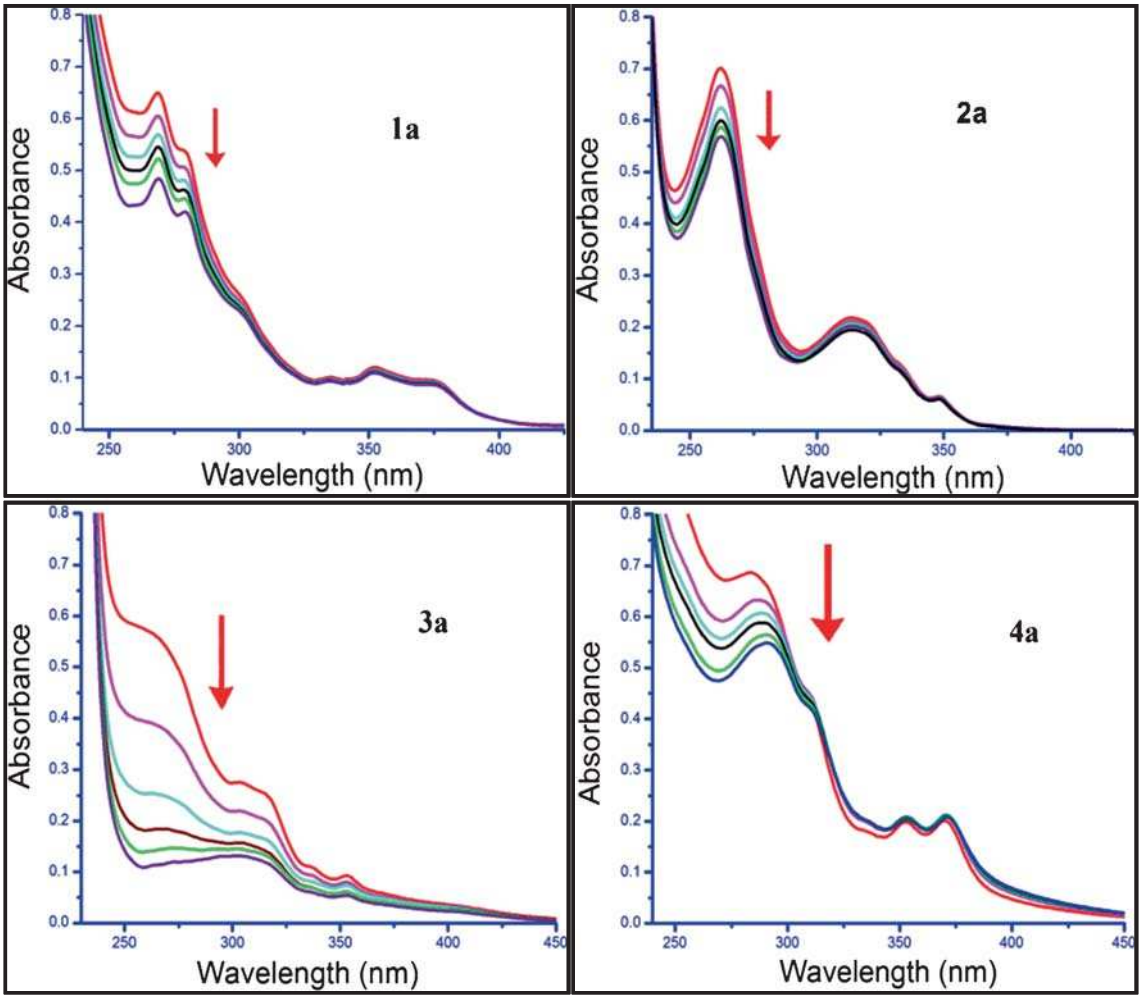


Figure 2. Changes in electronic absorption spectra of compounds **1a**-**4a** ($25x10^{-6}$ mol L^{-1}) with increasing concentrations ($0-25x10^{-6}$ mol L^{-1}) of CT-DNA. Arrow shows the absorption intensity decreases upon increase in DNA concentration.

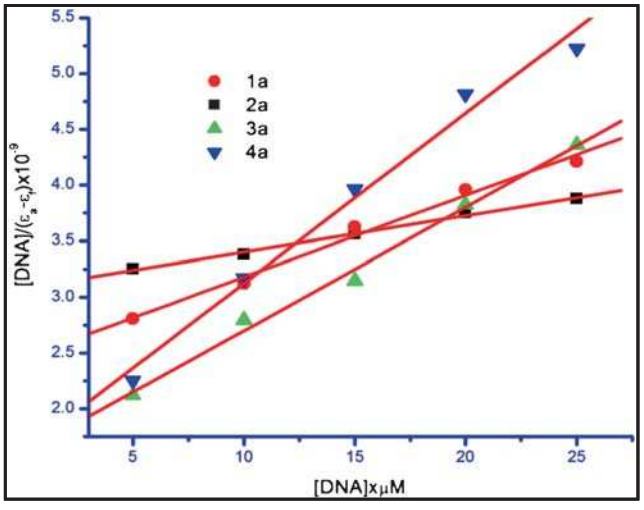


Figure 3. Plots of [DNA]/(ε_a-ε_f) versus [DNA] for the compounds **1a**-**4a** with CT-DNA. The unit along y-axis is (x 10^{-9} M^2 cm).

versus [DNA] gave a slope and intercept that are equal to $1/(\varepsilon_b - \varepsilon_f)$ and $1/Kb(\varepsilon_b - \varepsilon_f)$, respectively; Kb is the ratio of the slope to the intercept. The calculated intrinsic binding constants from absorption titration are shown in table 2.

Decreasing emission intensity of compounds **1a**-**4a** with incremental addition of CT-DNA (figure 4) in fluorescence studies suggest that they are bound to CT-DNA *via* an intercalative mode.[18]

In order to ascertain binding ability of the compound, competitive assay with ethidium bromide was carried out. Ethidium bromide is a well-known non-emissive intercalator but emissive when it binds with DNA. Tested compounds with various concentrations were added to DNA-EB mixture which causes various percentages of hypochromism and revealed that the newly synthesized compounds have the ability to displace EB from DNA-EB mixture. The fluorescence quenching spectra of the compounds showed a decrease in fluorescence intensity of the DNA-EB system with

Table 2. Binding Constant (K_b) of Compounds **1a**-**4a** in contact with CT-DNA.

Compound	λ_{max} (nm)	Hypochromicity (ΔE)	K_b (10^4 L mol^{-1})
1a	268	27.44	2.96± 0.07
2a	262	23.77	1.05± 0.02
3a	303	52.11	9.14± 0.16
4a	283	23.43	6.88± 0.08

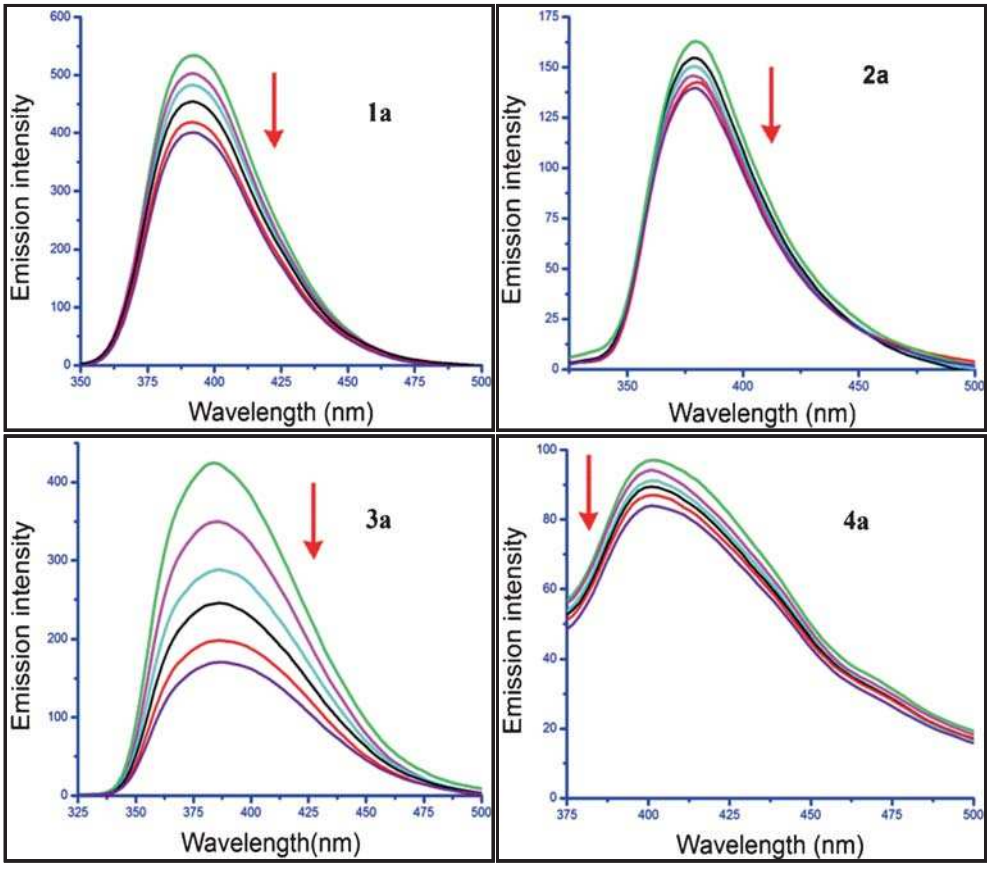


Figure 4. Emission enhancement spectra of compounds **1a**-**4a** (25x10^{-6} mol L^{-1}) with increasing concentrations (0-25x10^{-6} mol L^{-1}) of CT-DNA. Arrow shows the emission intensities decrease upon increasing DNA concentrations.

increasing concentration of the compounds (figure 5). The observed decrease in the fluorescence intensity indicates that the EB molecules are displaced from their DNA binding sites due to binding of **1a**-**4a** with DNA *via* intercalation. The quenching parameter can be calculated from the Stern−Volmer plots of I_o/I versus [Q] (figure 6), and the quenching constant (K_q) was calculated from the slope of the linear plot.

Quenching data were analyzed according to the Stern−Volmer equation: I0/I = Kq[Q] + 1, where I0 is the emission intensity in the absence of a quencher, I is the emission intensity in the presence of a quencher, Kq is the quenching constant, and [Q] is the quencher concentration. The Kq value is obtained as the slope from the plot of I0/I versus [Q]. The apparent binding constant (K_{app}) values were obtained using the following equation: KEB[EB] = Kapp[compound], where the compound concentration causes a 50% reduction of the fluorescence intensity of EB, KEB = 1.0 × 10^7 L mol^{-1}, and [EB] = 12x10^{-6} mol L^{-1}. The apparent binding constant (K_{app}) values (table 3) suggested stronger interaction of compounds with CT-DNA,[19] which is consistent with the absorption and emission observations.

Among the tested isomeric compounds of hydrazinylbenzo[*h*]quinolines, and isoindolin-1,3-diones, the binding constant (K_b), quenching constant (K_q) and apparent binding constant (K_{app}) values indicated

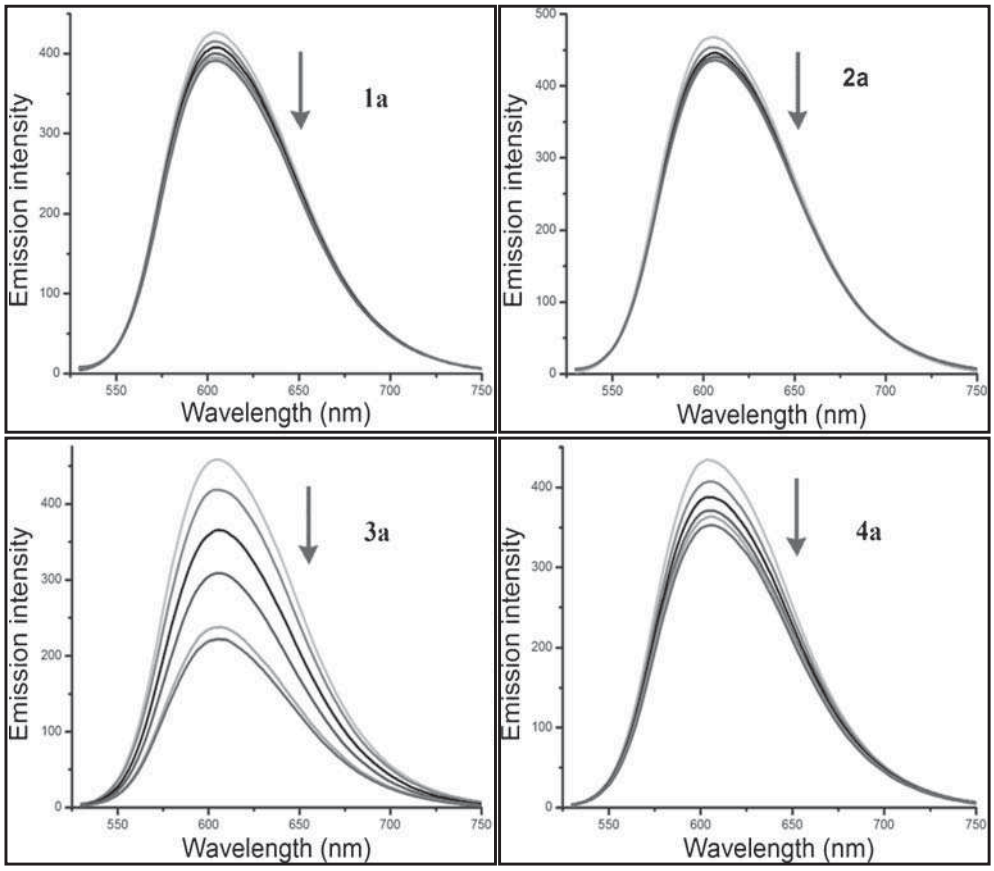


Figure 5. Fluorescence quenching curves of ethidium bromide ($12x10^{-6}$ mol L^{-1}) bound to CT-DNA ($12x10^{-6}$ mol L^{-1}) with increasing concentration of compounds **1a**-**4a** ($0\text{-}25x10^{-6}$ mol L^{-1}).

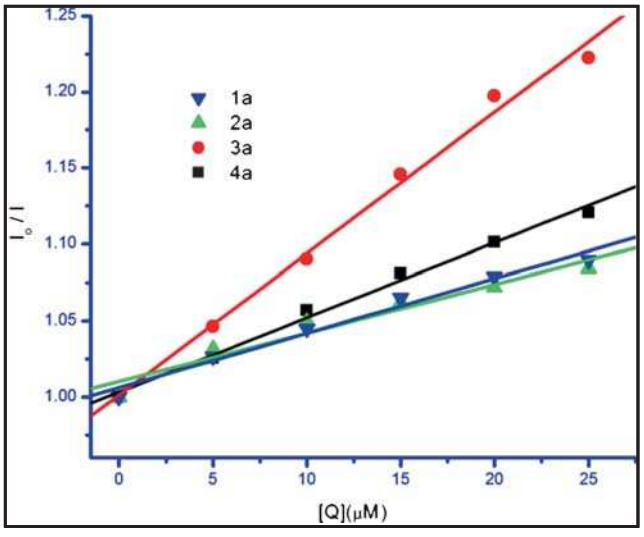


Figure 6. Stern−Volmer plots of the fluorescence titrations of compounds **1a**-**4a**.

in each case that the 4-substituted quinoline compounds have stronger DNA binding properties than the corresponding isomeric 2-substituted quinoline compounds. Isoindolin-1,3-diones showed more significant binding properties with DNA than hydrazinylbenzo[*h*] quinolines for both the 2- and 4-substituted compounds. The additional aromatic moiety in the isoindolin-1,3-diones facilitates increase binding affinity with double helix DNA.[20]

3.3 *Cytotoxicity*

The outcome of the significant results from DNA binding studies has encouraged us to test their *in vitro* anticancer activities against three human cancer cell lines such as human leukemia cell line (CCRF-CEM, ATCC no. CCL-119), Human colon adenocarcinoma (HT29, ATCC no. HTB-38) and human breast carcinoma (MCF7, ATCC no. HTB-22). The obtained results are summarized in table 4. All tested compounds showed moderate to better anticancer activity against tested cancer cell lines. The activities of starting compounds (**1**(**a**-**d**) and **2**(**a**-**d**)) were tested against the cell lines with IC_{50} in the range of 97.23–35.20 μmol L^{-1}. Very interestingly, compound **2c** showed very good activity with the IC_{50} of 35.20 ± 1.90 μmol L^{-1} against CCRF-CEM cancer cell line which is outranged

Table 3. Quenching constant (K_q) and apparent binding constant (K_{app}) of **1a – 4a** bound to CT-DNA.

Compound	Hypochromicity	K_q (10^3 L mol^{-1})	K_{app} (10^5 L mol^{-1})
1a	8.23	3.57± 0.04	4.28± 0.06
2a	4.07	3.18± 0.01	3.82± 0.03
3a	51.60	7.50± 0.01	9.00± 0.03
4a	18.21	6.73± 0.02	8.08± 0.04

Table 4. *In vitro* cytotoxicity.

Entry	CCRF-CEM	HT29	MCF7	Entry	CCRF-CEM	HT29	MCF7
1a	95.28± 3.35	94.93± 5.86	98.56± 0.39	**3a**	57.14± 4.20	84.48± 8.33	80.61± 0.54
1b	56.73± 2.09	29.42± 3.67	57.93± 1.07	**3b**	83.46± 7.89	86.32± 0.45	71.48± 0.37
1c	86.59± 6.52	40.49± 8.86	97.23± 7.63	**3c**	88.52± 4.97	98.83± 4.85	37.56± 1.96
1d	55.05± 1.51	72.23± 1.74	68.46± 1.46	**3d**	100.23± 2.31	101.81± 2.56	36.82± 2.80
2a	58.98± 1.84	93.32± 9.90	94.52± 3.52	**4a**	101.80± 8.85	105.64± 2.29	61.56± 0.76
2b	93.97± 4.35	63.84± 4.22	87.33± 1.80	**4b**	115.17± 1.05	103.02± 3.78	74.42± 1.42
2c	35.20± 1.90	91.78± 1.70	64.87± 2.64	**4c**	97.46± 5.50	102.00± 7.43	108.96± 3.24
2d	81.18± 1.97	92.91± 7.43	70.00± 4.39	**4d**	100.33± 2.01	99.67± 3.63	50.07± 1.33
DMSO	99.99± 2.42	100± 9.22	100± 4.81	**DOX**	43.83± 1.35	16.89± 1.72	33.75± 2.64

DMSO – Control, **DOX –** Doxorubicin, CCRF-CEM - Human leukemia, HT29 - Human colon adenocarcinoma, MCF7 - Human breast carcinoma

standard doxorubicin (43.83±1.32 μmol L^{-1}) and showed least activity against HT29 and MCF7 cell lines. Compounds **1b** and **1c** showed significant activity with the IC_{50} value of 29.42±3.67 and 40.49±8.86 μmol L^{-1} against HT29 cancer cell line which is quite comparable to the standard doxorubicin (16.89±1.72 μmol L^{-1}) and its isomeric counterparts **2b** and **2c** showed moderate activity. Compounds **1b** and **1d** showed noteworthy activity against MCF7 cell line with IC_{50} values of 57.93±1.07 and 68.46±1.46 μmol L^{-1} and its isomeric compounds **2b** and **2d** exhibited least activity. The compounds with hydrazine substitution at 4-position exhibited more activity than substitution of hydrazine in 2-position of the quinoline moiety, except the most potentially active compound **2c**.

In the next series of compounds (**3**(**a**-**d**) and **4**(**a**-**d**)), compound **3a** obtained from **1a** with phthalic anhydride showed moderate activity against CCRF-CEM cancer cell line with IC_{50} value of 57.14±4.20 μmol L^{-1} and showed least activity against HT29 and MCF7 cancer cell lines. Compounds **3c** and **3d** showed very good cytotoxicity against MCF7 cell line with IC_{50} value of 37.56±1.96 and 36.82±2.80 μmol L^{-1} and it is quite comparable with the standard doxorubicin (33.75±2.64 μmol L^{-1}). Compound **3b** showed least activity towards HT29 cell line. Compounds **4**(**a**-**d**) which were obtained from **2**(**a**-**d**) with phthalic anhydride showed moderate to weak anticancer activity against all the tested cell lines with the IC_{50} value ranging from 50.07±0.76 to 115.17±1.05 μmol L^{-1} respectively. In general, the results indicated that almost all the compounds showed reasonable cytotoxicity against MCF7 cell line and some of them were found to be selective against HT29 and CCRF-CEM cell lines. Furthermore, the compound containing methyl group on 8-position showed very good cytotoxic activity against all the tested cell lines.

4. Conclusions

We have developed a simple and efficient synthesis of aminoquinoline substituted isoindolin-1,3-diones using Eaton's reagent as a catalyst. The DNA binding properties of the compounds were investigated by absorption and fluorescence measurements. The results support that all the tested compounds bind to CT-DNA *via* intercalation. The binding affinity is greatly increased for the aminobenzo[*h*]quinoline substituted isoindolin-1, 3-diones when compared to hydrazinylbenzo[*h*]quinolines. All the synthesized compounds exhibited significant to moderate activity against MCF7 cell line than CCRF-CEM and HT29. The quinoline containing methyl substitution at 8-position highly enhances cytotoxicity against tested cancer cell lines. The compound 4-chloro-2-hydrazinyl-8-methylquinoline (**2c**) outranged the standard doxorubicin against CCRF-CEM cell line.

Supporting Information (SI)

Copies of ^{1}H and ^{13}C NMR spectra for all newly synthesized compounds are available at www.ias.ac.in/chemsci.

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