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Synthesis of Novel 1,3-Diacetoxy-Acridones as Cytotoxic Agents and their DNA-Binding Studies

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Abstract

A series of novel substituted acridones (**1–15**) have been synthesized. Their in vitro cytotoxicity against human breast adenocarcinoma (MCF-7) and human promyelocytic leukemia (HL-60) cell lines has been investigated. The compounds **11, 12, 14** and **15** showed moderate activity against MCF-7 cell lines with IC_{50} value < 5.83 μM. The compounds **8, 10–12,** and **15** showed moderate activity against HL-60 cell lines with IC_{50} value < 1.75 μM. The DNA-binding properties of the compounds were evaluated based on their affinity or intercalation with CT-DNA measured with absorption titration. The compound **12** bearing planar diacetoxy tricyclic ring linked with butyl piperidine side chain showed highest binding affinity with binding constant (Ki) 10.38 ×10 ×M^{-1}. The examination of the relationship between lipophilicity and cytotoxic properties of acridones showed a poor correlation.

Keywords

1,3-Diacetoxyacridone • Cytotoxicity • MCF-7 • HL-60, DNA-binding • Calf-Thymus DNA.

Introduction

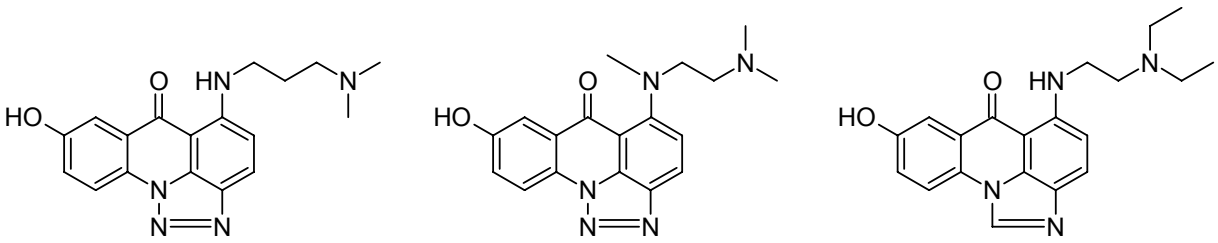
Cytotoxic drugs remain the mainstay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents [1]. Acronycine also known as acronine is an acridone alkaloid which was first isolated in 1948 from the bark of the Australian tree *Acronychia baurri* [2]. In subsequent investigation it was found that acronycine possess anticancer activity against a wide spectrum of experimental neoplasms in laboratory animals [3]. Glyfoline, another natural acridone alkaloid isolated from *Glycosmis citrifolia*, was found to be active molecule (IC_{50}=2.2 μM) for inhibition of human leukemic HL-60 cells [4, 5].

A series of thioacridone derivatives was synthesized by Dheyongera *et al.* The binding constant of these with DNA was measured to determine their degree of intercalation with DNA [6].

Triazoloacridone (C_{1305} and C_{1533}) (Fig. 1) binds to DNA and induces very specific and unusual structural changes in DNA, which plays an important role in the cytotoxic activity of this unique compound [7].

The imidazo acridones are a new class of antitumor agents. The most promising imidazo acridinone derivative C_{1311} (Fig. 1) is currently under phase II clinical trials for colon and breast cancers. C_{1311} damages cellular DNA in various ways. The drug binds to DNA non-covalently (by intercalation) and covalently, following oxidative metabolic activation [8].

The design of these compounds was based on structure activity relationship studies of the chemotherapeutic agent mitoxantrone. The diamino alkyl group in the side chain of mitoxantrone had previously been found to be prerequisite for biological activity of the drug. The attachment of diaminoalkyl group to known DNA-intercalating acridinone moieties resulted in the C_{13xx} imidazo acridinone series of the compounds which showed good activity in vitro and in vivo in various tumor model systems [9].



C_{1305} **C_{1533}** **C_{1311}**

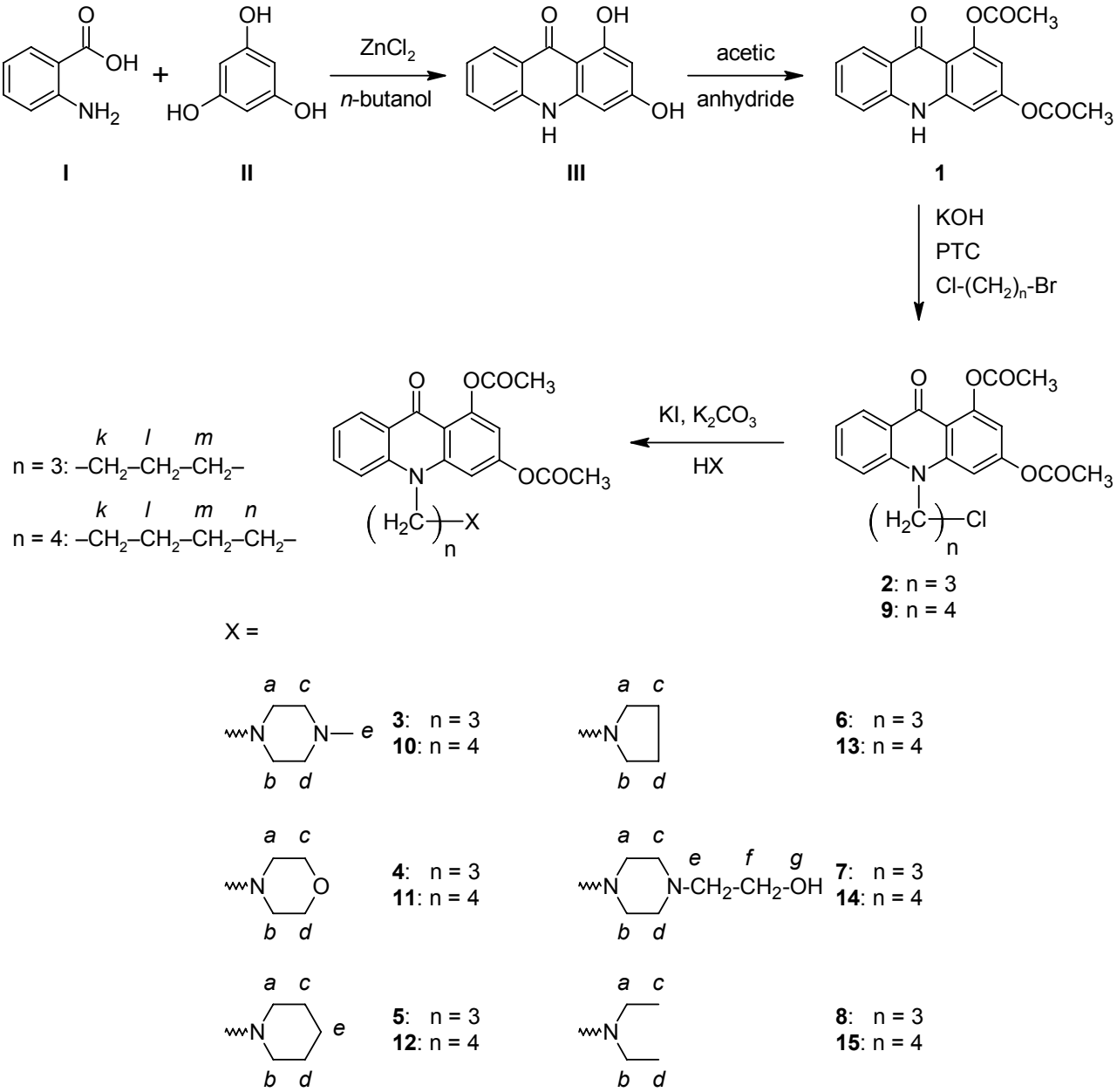
Fig. 1.

In the view of above literature, we have designed 1,3-diacetoxyacridone ring nucleus substituted at N^{10}-position with propyl and butyl side chains followed by tertiary amino groups for better cytotoxicity and DNA-binding properties (**1–15**, Tab. 1). Their cytotoxicity

has been tested against MCF-7 and HL-60 cell lines and tried to correlate the lipophilicity, DNA-binding properties with cytotoxicity.

Results and Discussion

Chemistry



Sch. 1.

1,3-Diacetoxyacridone **1** and its derivatives **2–15** were synthesized by the Sch. 1. Compound **1** was synthesized by cyclization of anthranilic acid and phloroglucinol using zinc chloride and followed by acetylation in quantitative yield.

Usually N-alkylation with alkyl halides is difficult due to the weak basic nature of nitrogen of the acridone nucleus. However, it can be achieved in the presence of a strong base like sodium amide or sodium hydroxide under anhydrous conditions. The reaction of parent acridone with chlorobromoalkanes in the presence of sodium amide in anhydrous condition gave respective N^{10}-(chloroalkyl)acridones. Besides requiring drastic experimental conditions, the N^{10}-alkylation using sodium amide resulted in a very low yield. To overcome this drawback, N^{10}-alkylation was carried out in the presence of phase transfer catalyst (PTC), which is easier to work with and gives better yield than the previously described methods.

Stirring of the compound **1** at room temperature with alkylating agent 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane in a two phase system consisting of an organic solvent and aqueous potassium hydroxide solution in the presence of tetra butyl ammonium bromide (PTC) leads to the formation of respective compounds **2** and **9** in good yield. Here, catalyst (PTC) transports the OH^- ion from the aqueous phase to organic phase where actual reaction takes place. The ion formed may be regarded as phenolate stabilized anion, which subsequently undergoes alkylation to form the aromatized system.

Iodide catalyzed nucleophilic substitution reaction of the N^{10}-chloropropyl or N^{10}-chlorobutyl 1,3-diacetoxy acridone with various secondary amines (*N*-methylpiperazine, piperidine, morpholine, (β-hydroxyethyl)piperazine, *N*,*N*-diethylamine and pyrrolidine) by refluxing for different time intervals in the presence of anhydrous potassium carbonate in acetonitrile gave the free bases **3–8** and **10–15.**

All the products were separated and purified by column chromatography and recrystallization method and dried under high vacuum for more than 12 h. The purified compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, Mass spectral methods and elemental analysis. The assignment of protons is fully supported by the integration curves and all the derivatives showed the characteristic chemical shifts for the acridone nucleus. The assignment of the ^{13}C-resonance of acridone derivatives is in close agreement with an analogous compound N^{10}-substituted acridone.

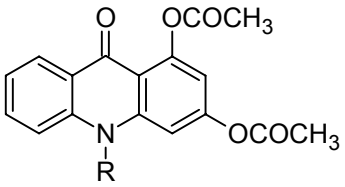
Biological activity

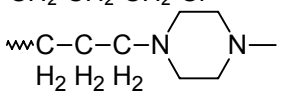
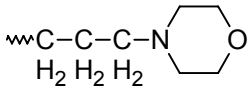
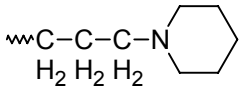
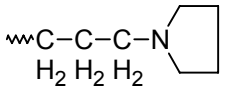
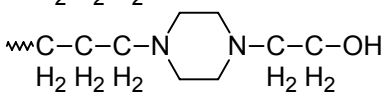
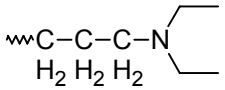
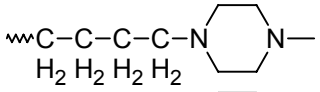
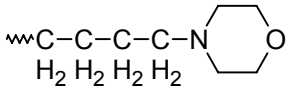
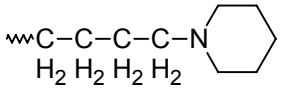
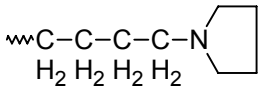
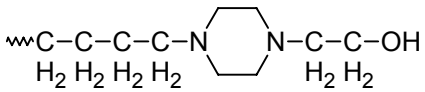
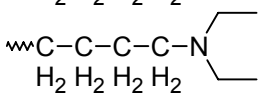
Lipophilicity

The compounds lipophilicity was determined using the software ALOGPS and $SPARC_{v4.2}$. The efficiency of a cytotoxic drug will depend in part on its ability to accumulate in cells. Lipophilicity of compounds plays a vital role in cytotoxicity effect of the compounds. The acridone derivatives are weak bases and able to exist in both charged (protonated) and uncharged (unprotonated) forms. The lipophilicity data of **1–15** varying from 2.83–4.06 and 2.02–4.43, expressed in $\log_{10}$P and logD respectively, are given in Tab. 1. Substitution of hydrogens by -$OCOCH_3$ in positions C-1 and C-3 and alkyl side chain with different tertiary amino groups resulted in a slight enhancement in the $\log_{10}$P and logD values. Additionally, the acridone nucleus with acetoxy groups at positions C-1 and C-3 and positively charged tertiary amino groups may exhibit higher affinity for membranes or more readily taken up into the cells. Analysis of the relationship between $\log_{10}$P and logD values and the cytotoxic activity in cancer cells showed poor correlation. The major outlier in this analysis was parent nucleus (**2** and **9**) are comparatively having higher $\log_{10}$P and logD values than

any of this substituted derivatives, yet these were not very effective at increasing cytotoxic activity. In contrast, compound **13** with $log_{10}P$ (3.88) and logD (4.01) values did not show maximum activity. However, compound **12** with $log_{10}P$ (4.06) and logD (4.43) showed maximum activity. Therefore, the degree of lipophilicity of each drug would seem to be important, but it is not the sole determinant for cytotoxicity of acridone derivatives.

Tab. 1. Lipophilicity values of different N-substituted Acridone Derivatives:



Comp. No.	R	$Log_{10}P$ [a]	LogD [b]
1	-H	3.20	2.01
2	$-CH_2-CH_2-CH_2-Cl$	3.60	3.75
3		2.83	2.91
4		2.95	2.66
5		3.89	3.96
6		3.92	3.62
7		2.87	2.59
8		3.66	3.75
9	$-CH_2-CH_2-CH_2-CH_2-Cl$	3.93	4.18
10		3.10	3.30
11		3.34	3.12
12		4.06	4.43
13		3.88	4.07
14		3.29	2.99
15		3.92	4.15

[a] $Log_{10}P$ values were calculated with ALOGPS software. [b] LogD values were calculated with SPARC v4.2 software.

Cytotoxic activity against MCF-7 and HL-60 cell lines

Tab. 2. Cytotoxicity of compounds **1–15** against MCF-7 and HL-60 cell lines:

Compounds	IC_{50} (μM) against MCF-7	IC_{50} (μM) against HL-60
1	36.70	—
2	28.78	16.45
3	16.65	15.39
4	10.24	13.71
5	11.98	14.06
6	15.12	14.59
7	9.84	10.90
8	14.56	1.65
9	24.44	14.25
10	8.98	1.74
11	4.97	0.51
12	3.15	1.41
13	9.34	14.11
14	4.24	11.01
15	5.82	0.45
Cyclophosphamide	0.16	—
Doxorubicin	—	0.020

The cytotoxicity of fifteen compounds was examined on MCF-7 and HL-60 cell lines by Trypan blue exclusion method with several concentrations of acridones. The IC_{50} values of N^{10}-chloropropyl substituted and chlorobutyl substituted 1,3-diacetoxy acridone derivatives against MCF-7 and HL-60 cells revealed that cytotoxic activity relatively increased as the chain length increased from three to four suggesting that hydrophobicity plays an important role in biological activity. The presence of acetoxy groups and increase of distance between the ring nucleus and amino group, increased the cytotoxic activity of these compounds. It is clear from the data, the comparison of the cytotoxicity against MCF-7 cell lines (Tab. 2) of the diacetoxy butyl derivatives has shown that the cell killing potency follows the order, **12 > 14 > 11 > 15 > 10 > 13 > 9** and diacetoxy propyl derivatives **7 > 4 > 5 > 8 > 6 > 3 > 2.** The cytotoxicity against HL-60 cell lines (Tab. 2) of the diacetoxy butyl derivatives has shown that the cell killing potency follows the order, **15 > 11 > 12 > 10 > 14 > 13 > 9** and diacetoxy propyl derivatives **8 > 7 > 4 > 5 > 6 > 3 >2.** However, comparison of IC_{50} values with in the series revealed that the diacetoxy butyl derivatives have higher potency than diacetoxy propyl derivatives. Among this series, the compound **12** showed moderate cytotoxic activity against MCF-7 cell line with IC_{50} value 3.15 μM and compound **15** against HL-60 with IC_{50} value 0.45 μM.

Therefore, it can be concluded that the structural features required with in the series to cause a maximum cytotoxic activity in MCF-7 and HL-60 cell lines, include hydrophobic acridone ring with electron withdrawing diacetoxy groups and alkyl side chain preferably four methylene units with substitution positively charged tertiary amino group preferably piperidino and diethyl amino groups.

DNA-binding properties

The DNA-binding properties of the compounds **3–5, 7, 8, 10**–**12, 14** and **15** (**1, 2, 6, 9** and **13** not analyzed due to poor solubility) were studied by monitoring the changes in the UV-Visible absorption spectra of the acridone derivatives up on addition of CT-DNA [10]. In the range from 260 to 275 all the acridone derivatives exhibited strong absorption peaks with maxima near 266-268nm. Progressive addition of DNA led to strong hypochromism in the absorption intensities in all the compounds studied. The percentage hypochromism were found to be 50.0, 58.6, 57.2, 62.6, 61.8, 54.1, 57.8, 53.5, 60.4 and 43.1. The Fig. 2 shows the representative absorption spectrum of the compound **11** (15 μM) in the presence of increasing concentration of CT-DNA (0-100 μM). The Fig. 3 shows Half- reciprocal plot for binding of **11** with CT DNA. The compound exhibited the similar absorption spectra pertaining to the chromophore but with the hypochromicity and isobastic point depending on the alkyl amino side chains. These results were consistant with the previous reports on the absorption titration of acridine derivatives and the hypochromicity of acridine in the presence of DNA is believed to be a result of their intercalation with the DNA [11].

The selection of ionic strength (150mM NaCl) in the absorption titration experiment was mainly based on the avoidance of DNA deposition in all drug solution (15μM). The Tab. 3 summarizes the DNA-binding constants and related properties of acridones after intercalation with CT-DNA. The relative binding affinities as indicated by the binding constant K_i were in the order of **12 > 14 > 11 > 4 > 10 > 5 > 7 > 15 > 3 > 8**. Among the derivatives those with strong DNA-binding affinities **11, 12** and **14** exhibited hypochromicity, isobastic points. However, a highest binding affinity and cytotoxic activity were observed for compound **12** bearing planar tricyclic ring with electron withdrawing diacetoxy groups, linked with butyl piperidine side chain.

Tab. 3. Binding constant (K_i) and photometric properties of acridones in contact with CT-DNA:

Compound	K_i (×10 ×M^{-1})	λ_{max} (nm)	Hypochromicity (%)	Isobestic point
3	1.41	266	50.0	296
4	3.42	268	58.6	334
5	2.78	268	57.2	Unclear
7	2.55	268	62.6	Unclear
8	0.71	268	61.8	290
10	2.8	268	54.1	320
11	4.12	268	57.8	325
12	10.38	266	53.5	336
14	8.04	266	60.4	Unclear
15	1.64	266	43.1	285

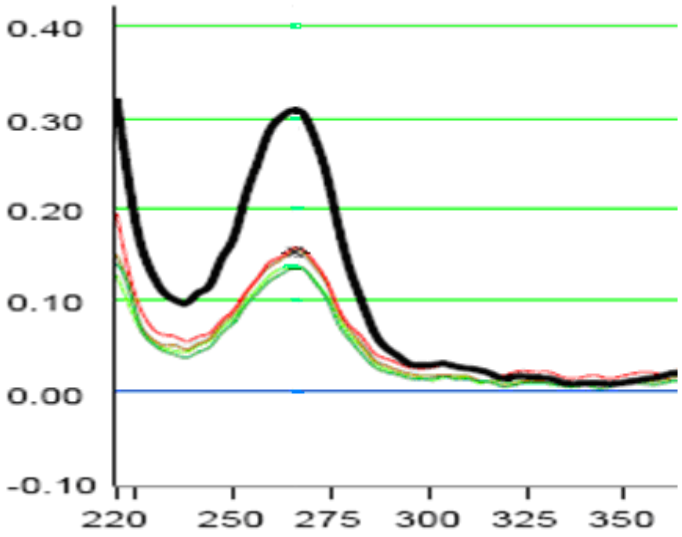


Fig. 2. Absorption titration of 11 at 15 μM in 20 mM sodium phosphate buffer (pH 6.5) with 150mM NaCl at Increasing CT DNA concentration .

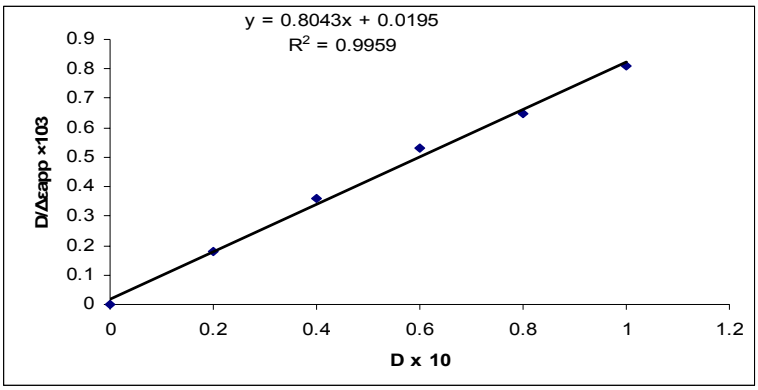


Fig. 3. Half-reciprocal plot for binding of 11 with CT DNA.

Conclusion

The new diacetoxyacridone derivatives derived from acridone with tertiary amines group at the terminal end of the alkyl side chains had strong inhibiting activity against MCF-7 and HL-60 cell lines, which may be associated with their DNA-binding capacity. In particular, the effect is more pronounced when acridones have propyl and butyl side chain. Comparison of the derivatives for their ability to bind with DNA revealed that they largely follow the order N^{10}-butyl side chain > N^{10}-propyl side chain. The substitution of hydrogens by $OCOCH_3$ increased the ability to bind DNA. Careful examination of the results obtained, revealed that the diacetoxy butyl derivatives have higher activity than diacetoxy propyl derivatives. With respect to these observations, we concluded that this series could be developed as a promising cytotoxicity as DNA-Intercalators.

Experimental

Reactions were monitored by TLC. Column Chromatography utilized silica gel Merck Grade 60 (230–400 mesh, 60 Å). Melting points were recorded on a Tempirol hot-stage with microscope and are uncorrected. Elemental analysis was performed and found values are ±0.4% of theoretical values unless otherwise noted. ^{1}H-NMR, ^{13}C-NMR spectra were recorded in $CDCl_3$, and DMSO-d_6 solution in a 5-mm tube on a Bruker DRX 400 Fourier transform spectrometer with tetramethylsilane as internal standard. Chemical shifts are expressed as δ (ppm) values. Mass spectra were recorded on Thermo Finnigan trace DSQ GC-Mass Spectrometer. DNA-binding studies of synthesized compounds were performed by Nano Drop ND-1000 UV Spectrophotometer.

Synthesis of 9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (1)

Compound **1** was synthesized by a method reported earlier [12].

General method for the synthesis of 10-(chlorobromoalkyl)-1,3-diacetoxy acridones (2) and (9)

One gram (0.0044 mol) of 1,3-diacetoxyacridone (**1**) was dissolved in 25 mL tetrahydrofuran and then 20 mL (0.05 mol) of potassium hydroxide and 0.5 g (0.015 mol) of tetrabutylammonium bromide was added to it. The reaction mixture was stirred at room temperature for 30 min and added Chlorobromoalkanes (0.015 mol) slowly into the reaction mixture and stirred for 24 h at room temperature. Tetrahydrofuran was evaporated and the aqueous layer was extracted with chloroform. The chloroform layer was washed with water and organic layer dried over anhydrous sodium sulfate and rotavaporated. The crude product was purified by column chromatography by using the solvent system chloroform/methanol (9:1) to give a green solid of (**2**) and (**9**).

10-(3-Chloropropyl)-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (2)

Yield: 55%, M.p. 150–155°C.^{1}H-NMR ($CDCl_3$) δ=7.39-8.33 (m, 6H, Ar-H), 2.05 (m, 2H, H_l), 3.99 (t, 2H, H_k, J=2H_Z), 3.55 (t, 2H, H_m, J=6Hz), 2.19 (s, 2$COCH_3$), ^{13}C-NMR ($CDCl_3$) δ=178.56 (C_9), 163.47 (2×OCO), 143.81 ($C_{9'}$,$C_{8'}$), 141.04 ($C_{10'}$,$C_{4'}$), 133.98 (C_7), 126.38 (C_4), 132.65 (C_5), 127.08 (C_6), 125.48 (C_2), 121.34 (C_8), 155.0 (C_1), 154.24 (C_3), 53.12 (C_k), 43.56 (C_l), 56.34 (C_m), 23.65 (C_1-CH_3), 21.58 (C_3-CH_3); MS *m/z*: 387.1 (M^+); 387.1(1.2), 385.0(2), 377.2(15), 376.1(64), 373.7(100), 318.0(22), 302.9(17), Anal. Calcd for $C_{20}H_{18}ClNO_5$: C, 61.94; H, 4.68; N, 3.61. Found: C, 61.89; H, 4.62; N, 3.58.

10-(4-Chlorobutyl)-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (9)

Yield 52%, Mp:120°C. ^{1}H-NMR ($CDCl_3$) δ=6.21-8.34 (m, 6H, Ar-H), 2.05 (m, 2H, H_l), 3.61 (t, 2H, H_k,J=6Hz), 4.21 (t, 2H, H_n J=8Hz), 1.6-2.26 (m, 4H, H_l and H_m), 2.3 (s, 6H, 2$COCH_3$); ^{13}C-NMR ($CDCl_3$) δ=178.68 (C_9), 163.49 (2×OCO), 143.46 ($C_{9'}$,$C_{8'}$), 142.00 ($C_{10'}$,$C_{4'}$), 133.58 (C_2), 132.24 (C_7), 128.06 (C_4), 126.09 (C_5), 123.43 (C_6), 121.84 (C_8), 155.05 (C_1), 154.64 (C_3), 53.82 (C_k), 52.49 (C_n), 24.29 (C_l), 23.28 (C_m) 22.52 (C_1-CH_3), 22.11 (C_3-CH_3); MS *m/z*: 400.9 (M^+); 400.9(8), 396.5(10), 374.0(26), 370.9(100), 330.0(20), 281.9(50), 91.0(35); Anal. Calcd for $C_{21}H_{20}ClNO_5$: C, 62.77; H, 5.02; N, 3.49. Found: C, 62.66; H, 4.98; N, 3.35.

*General procedure for the synthesis of 10-(*N*-substituted alkyl)-1,3-diacetoxy acridones (3–8 and 10–15)*

10-(Chloroalkyl)-1,3-diacetoxyacridone (0.0044 mol) was dissolved in 30 ml of anhydrous acetonitrile and 1.68 g potassium iodide and 3.3 g of potassium carbonate were added and refluxed for 30 min. Then added (0.0044 mol) different secondary amines into it slowly and refluxed for 15 h until a substantial amount of the product was formed as evidenced by TLC. The contents were cooled, diluted with water and extracted with chloroform. The chloroform layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product. The semi solid residue was purified by column chromatography using the solvent system chloroform/methanol (9:1) to give a light yellow product of 10-(3′-[*N*-substituted]alkyl)-1,3-diacetoxyacridone. An acetone solution of the free base was treated with ethereal hydrochloride to give the hydrochloride salt, which was dried over high vacuum to get pure solid.

10-[3-(4-Methylpiperazin-1-yl)propyl]-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (3)

Yield: 70%, M.p. 110°C.^{1}H-NMR (DMSO-d_6) δ=6.45-8.45 (m, 6H, Ar-H), 3.9-4.6 (m, 12H, H_k, H_m, H_a, H_b, H_c and H_d), 2.0-2.3 (m, 2H, H_l), 2.5, (s, 6H, 2$COCH_3$), 2.7 (s, 3H, H_e); ^{13}C-NMR (DMSO-d_6) δ=178.63 (C_9), 165.89 (2×OCO) 143.70 ($C_{9'}$,$C_{8'}$), 141.12 ($C_{10'}$,$C_{4'}$), 134.05 (C_2), 133.12 (C_7), 126.38(C_4), 125.60 (C_5), 123.20 (C_6), 121.35 (C_8), 155.42 (C_1), 154.16 (C_3), 57.98 (C_k), 52.52 (C_m), 49.40 (C_a), 48.00 (C_b), 43.33 (C_c,C_d), 26.54 (C_e), 24.29 (C_l), 23.75 (C_1-CH_3), 21.47 (C_3-CH_3); MS *m/z*: 452.2 (M+H); 452.2(12), 414.7(46), 384.5(50), 358.8(42), 306.6(30), 281.0(34), 206.8(100), 190.8(10), 73.0(14); Anal. Calcd for $C_{25}H_{29}N_3O_5$: C, 66.50; H, 6.47; N, 9.31. Found: C, 66.45; H, 6.25; N, 9.11.

10-(3-Morpholin-4-ylpropyl)-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (4)

Yield 83%, M.p. 75–80°C.^{1}H-NMR (DMSO-d_6) δ=6.4-8.3 (m, 6H, Ar-H), 3.1-4.5 (m, 12H, H_k, H_m, H_a, H_b H_c and H_d), 2.48 (m, 2H, H_l), 2.47 (s, 6H, 2$COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=177.54 (C_9), 165.90 (2×OCO), 155.12 (C_1), 154.5 (C_3), 141.15 ($C_{9'}$), 137.82 ($C_{8'}$), 135.60 ($C_{10'}$), 132.66 ($C_{4'}$), 120.70 (C_7), 129.69 (C_4), 125.87 (C_5), 120.70 (C_6), 123.26 (C_2), 118.08 (C_8), 54.19 (C_k), 51.49 (C_m), 58.19 (C_a, C_b), 63.35 (C_c, C_d), 26.17 (C_l), 20.68 (C_1-CH_3), 17.86 (C_3-CH_3); MS *m/z*: 438 (M^+); 438.0(8), 412(32), 356.8(50), 281.0(40), 266.4(68), 206.8(100), 177.1(38), 76.9(50); Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.61; H, 5.88; N, 6.28.

9-Oxo-10-(3-piperidin-1-ylpropyl)-9,10-dihydroacridine-1,3-diyl diacetate (5)

Yield 76%, M.p. 115°C. ^{1}H-NMR (DMSO-d_6) δ=6.5-8.35 (m, 6H, Ar-H), 3.2-4.4 (m, 8H, H_k, H_m, H_a and H_b), 1.3-2.0 (m, 8H, H_l, H_c and H_d and H_e), 2.5 (s, 6H, 2$COCH_3$) δ=176.54 (C_9), 175.78 (2×OCO), 154.64 (C_1), 150.73 (C_3),148.70 ($C_{9'}$), 143.79 ($C_{8'}$), 133.45 ($C_{10'}$), 132.66 ($C_{4'}$), 117.89 (C_7), 127.60 (C_4), 126.72 (C_5), 116.01 (C_6), 121.35 (C_2), 104.19 (C_8), 56.55 (C_k), 51.04 (C_m), 63.32 (C_a, C_b), 51.51 (C_c, C_d), 55.43 (C_l), 22.33 (C_e), 26.90 (C_1-CH_3), 20.47(C_3-CH_3); MS *m/z*: 436 (M^+); 436.0(8), 415.8(14), 354.6(39), 280.7(80), 207.1(100), 193.1(58), 135.9(40), 77.0(38); Anal. Calcd for $C_{25}H_{28}N_2O_5$: C, 68.79; H, 6.47; N, 6.42. Found: C, 68.75; H, 6.38; N, 6.38.

9-Oxo-10-(3-pyrrolidin-1-ylpropyl)-9,10-dihydroacridine-1,3-diyl diacetate (6)

Yield 60, M.p.80–84°C. ^{1}H-NMR (DMSO-d_6) δ=6.7-8.45 (m, 6H, Ar-H), 3.4-4.7 (m, 8H, H_k,

H_m, H_a and H_b), 1.8-2.1 (m, 6H, H_l, H_c and H_d), 2.5 (s, 6H, $2COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=178.25 (C_9), 166.80 (2×OCO) 143.48 ($C_{9'}$,$C_{8'}$), 133.72 ($C_{10'}$,$C_{4'}$), 141.11 (C_7), 126.88 (C_4), 126.11 (C_5), 123.10 (C_6), 121.08 (C_2), 120.65 (C_8), 156.96 (C_1), 155.10 (C_3), 54.79 (C_k, C_m), 45.56 (C_a, C_b), 23.90 (C_c, C_d), 24.64 (C_l), 21.92 (C_1-CH_3), 21.42 (C_3-CH_3); MS *m/z*: 422 (M^+); 422.0(6), 354.4(26), 281.2(30), 280.6(100), 266.6(32), 104.6(18), 83.9(98); Anal. Calcd for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63. Found: C, 68.33; H, 6.28; N, 6.54.

10-{3-[4-(2-Hydroxyethyl)piperazin-1-yl]propyl}-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (7)

Yield 88%, M.p. 90°C.^{1}H-NMR (DMSO-d_6) δ=6.6-8.4 (m, 6H, Ar-H), 3.1-4.15 (m, 14H, H_k, H_m, H_a, H_b, H_c, H_d and H_e), 2.2 (t, 2H, H_l, J=6Hz), 2.55 (s, 6H, $2COCH_3$), 4.3 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ=178.63 (C_9), 165.45 (2×OCO) 144.03 ($C_{9'}$,$C_{8'}$), 141.41 ($C_{10'}$, $C_{4'}$), 134.06 (C_2, C_7), 127.11(C_4), 126.42 (C_5), 123.30 (C_6), 121.40 (C_8), 155.62 (C_1), 155.01 (C_3), 58.14 (C_f), 55.73 (C_k, C_m), 48.42 (C_a, C_b), 43.68 (C_c, C_d), 28.54 (C_e), 26.82 (C_l), 24.53 (C_1-CH_3), 22.47 (C_3-CH_3); MS *m/z*: 481 (M^+); 481.0(8), 428.3(50), 355.5(48), 280.9(46), 207.0(100), 190.7(20), 96.1(18), 73.0(16); Anal. Calcd for $C_{26}H_{31}N_3O_6$: C, 64.85; H, 6.49; N, 8.73. Found: C, 64.75; H, 6.36; N, 8.59.

10-[3-(Diethylamino)propyl]-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (8)

Yield 75%, M.p. 85°C. ^{1}H-NMR (DMSO-d_6) δ=6.4-8.4 (m, 6H, Ar-H), 3.0-4.6 (m, 8H, H_k, H_m, H_a and H_b), 2.19 (m, 2H, H_l), 2.5 (s, 6H, $2COCH_3$), 1.0-1.45 (m, 6H, H_c and H_d); ^{13}C-NMR (DMSO-d_6) δ=178.14 (C_9), 166.32 (2×OCO), 143.60 ($C_{9'}$,$C_{8'}$), 142.78 ($C_{10'}$,$C_{4'}$), 133.51 (C_2), 132.7 (C_7), 125.28 (C_4), 126.92 (C_5), 125.92 (C_6), 120.98 (C_8), 156.85 (C_1), 155.18 (C_3), 58.08 (C_k), 57.92 (C_m), 67.52 (C_a, C_b), 8.47 (C_c, C_d), 30.01 (C_l), 24.18 (C_1-CH_3), 21.58 (C_3-CH_3); MS *m/z*: 424 (M^+); 424.0(50), 400.9(60), 376.8(30), 280.9(40), 207.0(100), 132.9(14), 95.6(10), 72.9(20); Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.99; H, 6.72; N, 6.71.

10-[3-(4-Methylpiperazin-1-yl)butyl]-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (10)

Yield 80%, M.p. 110°C. ^{1}H-NMR (DMSO-d_6) δ=6.2-8.3 (m, Ar-H), 3.1-4.5 (m, 12H, Hk, Hn, Ha, Hb, Hc and Hm), 1.5-2.0 (m, 4H, H_l and Hm), 2.85 (s, 3H, He), 2.55 (s, 6H, $2COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=178.57 (C_9), 165.80 (2×OCO) 143.36 ($C_{9'}$,$C_{8'}$), 141.00 ($C_{10'}$,$C_{4'}$), 133.45 (C_2), 132.12 (C_7), 127.05(C_4), 126.08 (C_5), 123.32 (C_6), 120.84 (C_8), 155.50 (C_1), 154.28 (C_3), 53.98 (C_k), 52.52 (C_n), 45.67 (C_a, C_b, C_c, C_d), 24.32 (C_e), 24.11 (C_l), 23.02 (C_m) 22.62 (C_1-CH_3), 22.05 (C_3-CH_3); MS *m/z*: 464.3 (M^+); 464.3(38), 421.9(100), 350.0(84), 281.1(40), 207.0(70), 112.9(24), 96.0(8); Anal. Calcd for $C_{26}H_{31}N_3O_5$: C, 67.08; H, 6.71; N, 9.03. Found: C, 66.99; H, 6.63; N, 8.59.

10-(3-Morpholin-4-ylbutyl)-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (11)

Yield 81%,. M.p. 98° C. ^{1}H-NMR (DMSO-d_6) δ=6.15-8.35 (m, 6H, Ar-H), 3.0-4.5 (m, 12H, Hk, Hn, Ha, Hb, Hc and Hd), 1.5-2.2 (m, 4H, Hl and Hm), 2.5 (s, 6H, $2COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=177.21 (C_9), 165.90 (2×OCO), 155.14 (C_1), 154.50 (C_3), 141.15 ($C_{9'}$), 138.90 ($C_{8'}$), 137.60 ($C_{10'}$), 135.2 ($C_{4'}$), 121.05 (C_7), 133.50 (C_4), 131.5 (C_5), 124.09 (C_6), 120.5 (C_2), 119.20 (C_8), 54.19 (C_k), 51.49 (C_n), 56.13 (C_a, C_b), 63.51 (C_c, C_d), 25.01 (C_l, C_m), 20.48 (C_1-CH_3), 17.93 (C_3-CH_3); MS *m/z*: 452.1 (M+H); 452.1(10), 370.9(8), 356.1(28), 280.9(50), 193.0(64), 132.7(90), 72.9(100), 69.0(28), 56.9(20); Anal. Calcd for $C_{25}H_{28}N_2O_6$: C, 66.36; H, 6.24; N, 6.19. Found: C, 66.25; H, 6.11; N, 6.10.

9-Oxo-10-(3-piperidin-1-ylbutyl)-9,10-dihydroacridine-1,3-diyl diacetate (12)

Yield 65%. M.p. 65-70° C. ^{1}H-NMR (DMSO-d_6) δ= 6.7-8.35 (m, 6H, Ar-H), 3.1-4.4 (m, 8H, Hk, Hn, Ha, Hb, Hc and Hd), 1.6-2.1 (m, 8H, Hl, Hm, Ha and Hd), 1.35 (m, 2H, He) 2.5 (s, 6H, 2$COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=178.54 (C_9), 176.5 (2×OCO), 154.96 (C_1), 154.55 (C_3), 148.70 ($C_{9'}$), 144.14 ($C_{8'}$), 139.51 ($C_{10'}$), 133.56 ($C_{4'}$), 117.89 (C_7), 126.54 (C_4), 121.42 (C_5), 120.31 (C_6), 121.45 (C_2), 104.1 (C_8), 56.55 (C_k), 53.37 (C_n), 57.11 (C_a, C_b), 27.42 (C_c, C_d), 51.04 (C_l, C_m), 22.61 (C_1-CH_3), 22.13 (C_3-CH_3), 21.33 (C_e); MS *m/z*: 449.8 (M^+); 449.8(8), 431.0(20), 392.0(100), 280.8(42), 206.9(90), 132.9(10), 96.0(8); Anal. Calcd for $C_{26}H_{30}N_2O_5$: C, 69.31; H, 6.71; N, 6.22. Found: C, 69.38; H, 6.60; N, 6.29.

9-Oxo-10-(3-pyrrolidin-1-ylbutyl)-9,10-dihydroacridine-1,3-diyl diacetate (13)

Yield 60%. M.p. 95°C.^{1}H-NMR (DMSO-d_6) δ=6.2-8.35 (m, 6H, Ar-H), 3.0-4.5 (m, 8H, Hk, Hn, Ha and Hb), 1.1-2.2 (m, 8H, H_l, Hm, Hc, and Hd), 2.5 (s, 6H, 2$COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=178.28 (C_9), 166.01 (2×OCO) 143.58 ($C_{9'}$,$C_{8'}$), 133.78 ($C_{10'}$,$C_{4'}$), 141.10 (C_7), 126.86 (C_4), 126.10 (C_5), 123.04 (C_6), 121.07 (C_2), 120.64 (C_8), 156.81 (C_1), 155.84 (C_3), 54.80 (C_k, C_n), 45.58 (C_a, C_b), 23.90 (C_c, C_d), 24.33 (C_l, C_m) 21.91 (C_1-CH_3), 21.41 (C_3-CH_3); MS *m/z*: 436.7 (M + H^+); 436.7(10), 412.7(22), 391.9(68), 325.1(50), 280.9(48), 190.8(14), 84.0(62), 73.0(20); Anal. Calcd for $C_{25}H_{28}N_2O_5$: C, 68.79; H, 6.47; N, 6.42. Found: C, 68.65; H, 6.35; N, 6.50.

10-{4-[4-(2-Hydroxyethyl)piperazin-1-yl]butyl}-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (14)

Yield 55%, M.p. 120°C. ^{1}H-NMR (DMSO-d_6) δ=6.15-8.35 (m, 6H, Ar-H), 3.1-4.5 (m, 16H, Hk, Hn, Ha, Hb, Hc, Hd He and Hf), 1.5-2.2 (m, 4H, Hl and Hm), 2.5 (s, 6H, 2 $COCH_3$), 5.4 (s, broad), 1H, OH; ^{13}C-NMR (DMSO-d_6) δ=178.53 (C_9), 165.36 (2×OCO) 143.66 ($C_{9'}$, $C_{8'}$), 141.11 ($C_{10'}$, $C_{4'}$), 133.96 (C_2), 133.07 (C_7), 127.09 (C_4), 126.50 (C_5), 121.25 (C_6), 118.51 (C_8), 155.42 (C_1), 155.08 (C_3), 55.53 (C_f), 48.44 (C_e), 45.75 (C_k, C_n), 24.45 (C_c, C_d), 24.45 (C_a, C_b), 24.05 (C_l, C_m), 21.15 (C_1-CH_3), 20.65 (C_3-CH_3); MS *m/z*: 494.8 (M^+); 494.4(8), 436.8(36), 380.0(86), 206.9(100), 112.9(48), 84.0(70); Anal. Calcd for $C_{27}H_{33}N_3O_6$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.35; H, 6.60; N, 8.32.

10-[3-(Diethylamino)butyl]-9-oxo-9,10-dihydroacridine-1,3-diyl diacetate (15)

Yield 98%, M.p. 85°C. ^{1}H-NMR (DMSO-d_6) δ=6.3-8.3 (m, 6H, Ar-H), 4.2 (t, 2H, H_k, J=8Hz), 2.8 (m, 6H, H_n, H_a and H_b), 1.8 (m, 4H, H_l and H_m) 2.55 (s, 6H, 2$COCH_3$); ^{13}C-NMR (DMSO-d_6) δ=178.60 (C_9), 165.82 (2×OCO) 143.86 ($C_{9'}$,$C_{8'}$), 141.38 ($C_{10'}$,$C_{4'}$), 134.01 (C_7), 126.32 (C_4), 133.11 (C_5), 127.10 (C_6), 123.30 (C_2), 121.29 (C_8), 155.92 (C_1), 155.20 (C_3), 46.60 (C_k), 45.82 (C_n), 50.74 (C_a, C_b), 8.82 (C_c, C_d), 24.57 (C_l, C_m), 22.12 (C_1-CH_3), 21.58 (C_3-CH_3); MS *m/z*: 438.7 (M+H); 438.7(12), 394.8(44), 367.7(66), 280.8(50), 206.9(100), 190.9(10), 97.9(14), 83.9(72); Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.36; H, 6.99; N, 6.48.

Cytotoxicity assay against MCF-7 and HL-60 cell lines

The trypan blue dye exclusion test was used to determine drug-mediated cytotoxicity as described previously [13]. Briefly, 1 x 10^4 target tumor cells resuspended in 1 ml. Two ml of cell suspension were distributed into each well of a 6-well plate, and medium at the desired concentration was added into each well. Each plate was incubated for 48 h at 37°C and 5% CO_2 atmosphere. Following the incubations, 100 µl of the trypan was added

into 100 µl of cell suspension. After this process, viable and dead cells were counted and percentage cytotoxicity was calculated. The IC_{50} was determined from concentration percentage cytotoxicity curve.

DNA binding assay by absorption titration

The spectrometric titration was conducted by Nano Drop ND-1000 UV Spectrophotometer at room temperature (~30°C). The CT DNA (Sigma, St. Louis, MO) was dissolved in double distilled de-ionized water with 50 mM NaCl, and dialyzed against a buffer solution for 2 days. Its concentration was determined by absorption spectrometry at 260 nm using a molar extinction coefficient 6600 M^{-1} cm^{-1}. The ratio A260/A280 > 1.80 was used as an indication of a protein-free DNA. Absorption titration was performed at a fixed concentration of drugs (15 µM) in a sodium phosphate buffer (20 mM sodium phosphate, 150 mM NaCl, pH 6.5). Small aliquots of concentrated CT DNA (3.9 mM) were added into the solution at final concentrations from 0 to 100 µM, and stirred for 5 min before measurement. The parameters, λmax, hypochromicity, isobastic point and binding constant were found from the absorption spectra. The intrinsic binding constant (K_i) [14] for a given complex with DNA was obtained from a plot of D/Δεapp versus D according to equation, $D/\Delta\varepsilon_{app} = D/\Delta\varepsilon + 1/\Delta\varepsilon \times K$, Where D= concentration of DNA in base molarities, $\Delta\varepsilon_{app} = |\varepsilon_a - \varepsilon_f|$ and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$, Where ε_a and ε_f are respective extinction coefficient of the complex in the presence and absence of DNA. The apparent extinction coefficient ε_a is obtained by calculating Aobs/ [Acridones]. The data were fitted to the equation with a slope equal to $1/\Delta\varepsilon$ and Y-intercept equal to $1/(\Delta\varepsilon \times K)$. The intrinsic binding constant (K_i) is determined from the slope of Y-intercept.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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