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DESIGN, SYNTHESIS, DNA-BINDING AND DOCKING STUDY OF 2,4-DIMETHOXY ACRIDONES AS CYTOTOXIC AGENTS

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Keywords:

2,4-Dimethoxy acridone, DNA-binding, Calf-Thymus DNA, Docking, HPLC and Lipophilicity

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ABSTRACT: A series of novel N^{10} -substituted 2,4-Dimethoxy acridones were synthesized as potent cytotoxic agents. DNA-binding studies of the synthesized compounds were carried out by the HPLC method using Calf thymus DNA (CT - DNA) based on their affinity or intercalation with CT-DNA, measured by binding percentage. The compound 12 bearing planar dimethoxy tricyclic ring linked with butyl pyrazole side chain showed the highest percentage of binding (90.78%) with CT-DNA and showed binding affinity of -6.08 Kcal/mol with the lipophilicity value of 3.33. Compound 8 showed the highest lipophilicity value of 5.09 with a binding affinity of -6.04 Kcal/mol and a percentage of drug binding (80.93%). Compound 5 showed high binding affinity of 7.01 Kcal/mol with percentage of drug binding (66.94%) and lipophilicity value of 3.72. The Binding affinity and percentage of drug binding showed favorable results. The docking outcome was in good agreement with DNA-binding results confirming the DNA binding activity of compounds. The lipophilicity and binding ability of the acridones with CT-DNA showed poor correlation.

INTRODUCTION: Cancer is a challenging disease with the second-highest mortality rate [1]. The attractive target for the design of new anti-tumor agents is DNA. There is a considerable interest taken in the development of new drugs with low molecular weight to bind DNA for their application in cancer treatment. It has been found that many cytotoxic drugs interact with DNA in different ways; a) Intercalation or through minor groove binding b) Cross-linking or combination of these modalities [2]. Several planar molecules containing tricyclic structures with different side chains have been reported to possess significant cytotoxic and cytostatic potencies. These tricyclic systems include anthraquinone, acridine and xanthene.

Several acridone derivatives with or without an alkyl side chain connected to the N^{10}-position have exhibited anti-cancer properties. Another imidazo acridone derivative has excellent anti-cancer and anti-MDR properties against a number of human cancer cell lines; studies have shown that the activity is due to DNA intercalation [3].

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Sathish N. K. *et al.* synthesized 1,3-dimethyl acridones [4] and 1,3-diacetoxy acridones, and their DNA-binding studies were carried out by absorption titration using CT-DNA. The compounds with acetoxy groups showed good binding properties compared to methyl groups [5]. The DNA-binding properties of 2-nitro acridone (NAD) and its analogs were carried out using salmon sperm DNA by electrochemical and CT-DNA by absorption titration method. The compounds showed good binding properties with DNA. This showed that electron-withdrawing groups attached to the acridone nucleus help in improving the binding ability of acridones towards DNA [6-7].

Acridone scaffolds' importance, effective anticancer agents specific to a cancer cell, capable of multiple binding targets and devoid of acquiring drug resistance, needs to be synthesized. However, from the literature survey, it was believed that the anticancer activity is due to their DNA intercalation property.

DNA binding studies help to determine the binding ability of different derivatives towards DNA. Higher the binding ability, will be the cytotoxicity of the drug. In the present study, an attempt was made to design and synthesize novel 2, 4-dimethoxy acridones for their DNA- binding properties and correlated with docking and Lipophilicity data.

MATERIALS AND METHODS:

Experimental Section: Progress of reactions was monitored by TLC. Melting points were recorded using Thiele's tube apparatus. Proton magnetic resonance (^{1}H-NMR) was obtained in DMSO-d6 and $CDCl_3$ on a Bruker drx 500 fourier transform spectrometer with tetramethylsilane as the internal standard. Chemical shifts are expressed as δ (ppm) values. The spectrometer was internally locked to the deuterium frequency of the solvent.

Acridone derivatives were also characterized by Shimadzu LC-MS spectrometer to know the molecular weight. Molecular docking studies of active cytotoxic analogs were performed by Auto dock 4.2 virtual screening software running on intel core i3 windows 7 operating system. The DNA-binding properties of the synthesized compounds were studied by HPLC Shimadzu LC-20A.

Synthesis of 2, 4-Dimethoxy Diphenylamine-2-Carboxylic Acid (Ullmann Condensation): Mixture of o-Chlorobenzoic acid (5 g, 0.032 mol), 2,4-Dimethoxyaniline (5 ml, 0.032 mol), copper powder (0.2 g) and 30 ml isoamyl alcohol were taken in a three-necked round bottom flask, anhydrous potassium carbonate (5 g) was added slowly with stirring. Refluxed for 8 h on an oil bath, steam distilled to remove isoamyl alcohol. The mixture was poured into 1 litre of hot water, acidified with concentrated hydrochloric acid.

The precipitate formed was filtered, washed with hot water, and dissolved in an aqueous sodium hydroxide solution, boiled in the presence of activated charcoal, and filtered. On acidification of the filtrate with concentrated hydrochloric acid, the precipitate obtained was washed with hot water and recrystallized from methanol to give a yellow solid (yield 85%), m.p. 180-185°C.

Synthesis of 2,4-Dimethoxy Acridin-9(10H)-one (1): 2,4-Dimethoxy diphenylamine-2- carboxylic acid (5.46g, 0.02 mol) taken in a flask to which polyphosphoric acid (50 g) was added, stirred well and heated on a water bath for 4 h. The appearance of yellow color indicated the completion of the reaction. Then, the mixture was poured into 1 litre of hot water and basified with liquor ammonia and yellow precipitate obtained was filtered, washed with hot water and recrystallized from acetic acid.

Yield: 60%, M.p. 210-215°C. FTIR (V cm-1, KBr): 3333 (N-H), 2869 (O-CH3) 1656 (C=O), 1581 (C=C), 1267 (C-N). 1H NMR (DMSO-d6): δ 8.5 (s, 1H, cyclic NH), 6.8-7.6 (m, 6H, Ar-H), 3.9 and 4.1 [s,s, 6H, $(OCH_3)2$].

Synthesis of 10- (Chloroalkyl)-2,4-dimethoxy acridin-9(10H)-one: 2,4-Dimethoxy acridone (1g, 0.0042 mol) dissolved in tetrahydrofuran (25 ml) in three-necked round bottom flask and 6N potassium hydroxide (17.5 ml) and tetrabutylammonium bromide (0.43 g, 0.0013 mol) was added to it.

The reaction mixture was stirred for 30 min at room temperature, 1-bromo-3chloropropane (2.04g, 0.013 mol) / 1-bromo-4-chlorobutane (2.22g, 0.013 mol) was added 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 dried over anhydrous sodium sulfate and rotavaporated. The crude product was purified by column chromatography by using the solvent system chloroform/ acetone (8:1) to give a yellow-colored product.

10-(3-Chloropropyl)-2,4-dimethoxy acridin-9(10H)-one: Yield: 50%, ^{1}H NMR (CDCl3) δ 8.3-7.2(m, 2 6H, Ar-H), 3.8 and 3.6 [s,s, 6H, $(OCH_3)2$], 3.4-3.2 (t, 2H, CH_2), 3.0 - 2.7 (t, 2H, CH_2), 2.0-1.8 (m, 2H, CH_2). MS m/z: 332.79(M+1).

10-(4-Chlorobutyl)-2,4-dimethoxy Acridin-9(10H)-one: Yield: 52%, ^{1}H NMR (CDCl3) δ 7.4-6.5(m, 6H, Ar-H), 3.6 and 3.4[s,s, 6H, $(OCH_3)2$], 3.3-3.1 (t, 2H, CH_2), 2.6-2.4(t, 2H, CH_2), 1.8-1.6 (m, 4H, CH_2 CH_2). MS m/z: 346.79(M+1).

General Method for the Synthesis of Derivatives (2-13): 10-(Chloroalkyl)-2,4-dimethoxy acridin-9(10H)-one (0.005 mol) was dissolved in 60 ml of anhydrous acetonitrile in three-necked round bottom flask, and 1.68g of potassium iodide and 3.3 g of potassium carbonate were added and refluxed for 30 min.

The various secondary amines (0.009 mol) were added slowly, refluxed for 15 hrs, and the contents were cooled, diluted with water, and extracted with chloroform.

The chloroform layer was washed three times with water. The organic layer was dried over anhydrous sodium sulfate and evaporated to give product and purified by column chromatography using chloroform and methanol (9:1).

2, 4 - Dimethoxy – 10 - (4-morpholino butyl) Acridine - 9(10H)-one(2): Yield: 50%, M.p. 125-127°C. ^{1}H NMR (DMSO-d6) δ= 7.5- 6.5(m, 6H, ArH), 4.1and 3.9[s, 6H, $(OCH_3)2$]. 3.8-3.5 (t, 4H, CH_2 CH_2), 3.3-3.0(t, 2H, CH_2) 2.8-2.6 (t, 4H, CH_2 CH_2), 2.4-2.3 (t, 2H, CH_2), 1.5-1.3 (m, 4H, CH_2 CH_2). MS m/z: 397.47 (M+H).

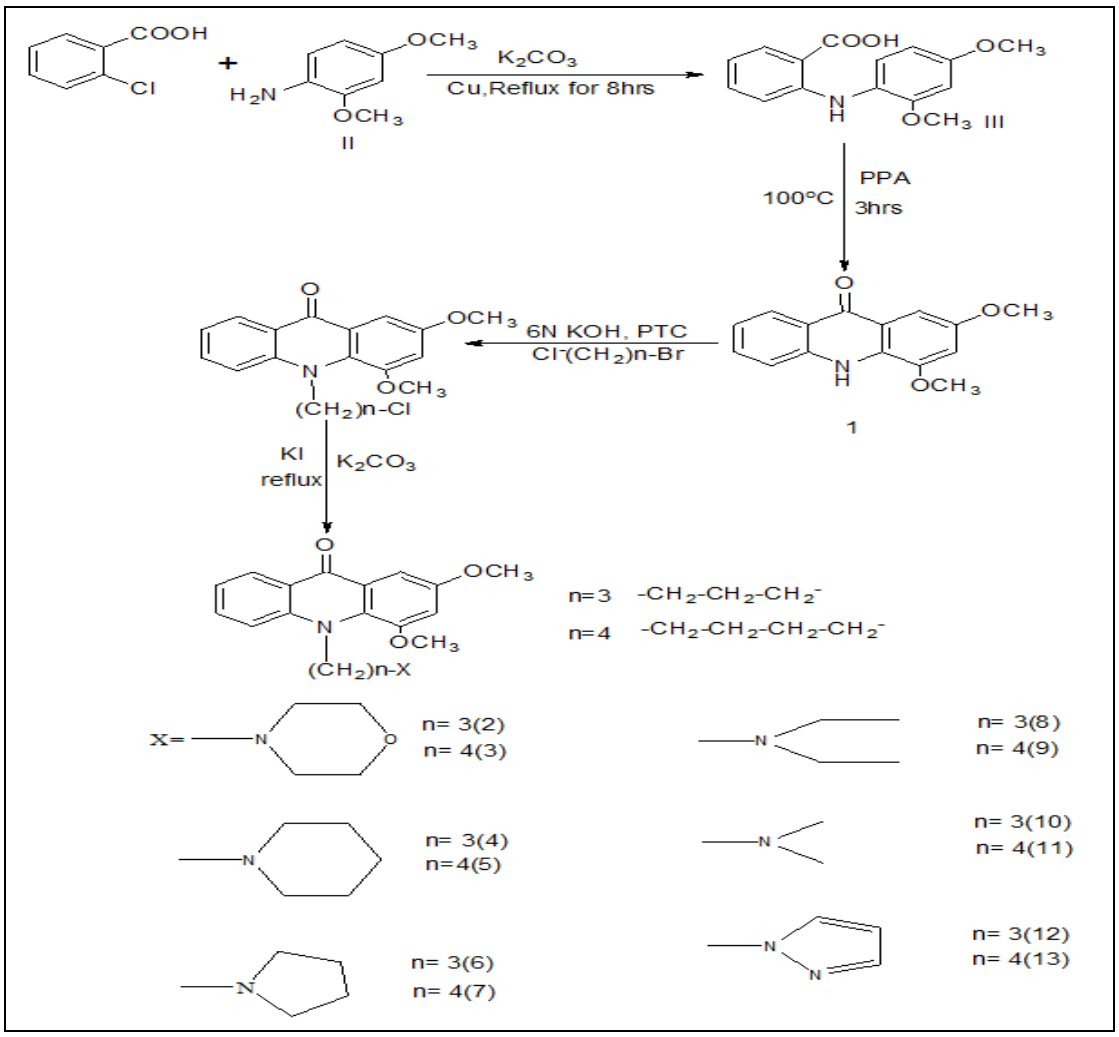
2, 4 – Dimethoxy – 10 - (3 -morpholinopropyl) Acridine - 9(10H)-one (3): Yield: 45%, M.p.83-85°C. ^{1}H NMR (DMSO-d6) δ= 8.2-6.5 (m, 6H, Ar-H), 4.0 and 3.8[s,s, 6H, $OCH_3)2$], 3.6-3.0 [m, 8H, $(CH_2)4$], 2.8-2.1(m, 8H, $(CH_2)4$]. MS m/z: 383.67 (M+H).

RESULTS AND DISCUSSION:

Chemistry: 2, 4-Dimethoxy acridin-9(10H)-one (1) and its derivatives 2-13 were synthesized by the scheme-1. *o*-Chloro benzoic acid reacts with 2,4-Dimethoxy aniline by the Ulmann condensation reaction to form 2,4-Dimethoxy diphenylamine-2-carboxylicacid (III). It undergoes the process of cyclization by refluxing with polyphosphoric acid instead of sulfuric acid on a water bath to get the single product of 2,4-Dimethoxy acridone (1) with a good yield of 95%. 2,4-Dimethoxy acridone was subjected to N-alkylation by using tetra butyl ammonium bromide (PTC), because the nitrogen atom of the acridone nucleus is generally resistant to undergo N-alkylation with alkyl halides due to the weakly basic nature of nitrogen.

2,4-Dimethoxy acridone (1) stirred with alkylating agents 1- Bromo-3-chloro propane or 1-Bromo-4-chloro butane in a two-phase system consisting of an organic solvent (tetrahydrofuran) and 6N aqueous potassium hydroxide solution in the presence of tetra butyl ammonium bromide (PTC) at a room temperature to synthesize the compounds 10-(3'-Chloropropyl)-2,4-dimethoxy acridone and 10-(4'- Chlorobutyl)-2,4-dimethoxy acridone respectively in good yields. Here, catalyst (PTC) carries the OH^- ion from the aqueous phase to an organic phase where the actual reaction occurs. Results in the formation of phenolate stabilized anion, which subsequently undergoes alkylation to form the aromatized system.

N^{10}-Chloropropyl or N^{10}-Chlorobutyl-2,4-dimethoxy acridone undergoes nucleophilic substitution with various secondary amines (piperidine, morpholine, pyrrolidine, diethylamine, dimethylamine and pyrazole) using potassium iodide as a catalyst by refluxing for different time intervals in the presence of anhydrous potassium carbonate in acetonitrile gave the free bases 2-13. All the compounds were purified by recrystallization method and dried under a high vacuum for more than 12 h. The compounds were characterized by ^{1}H-NMR and Mass spectrometry. The integration curves fully support the assignment of protons and all the derivatives showed the characteristic chemical shifts for the acridone nucleus.



SCHEME 1:

Docking Study: Ligand-nuclear receptor interaction is one of the important steps, molecular docking studies of active cytotoxic analogs (2-13) were performed by Auto Dock 4.2 virtual screening software running on intel core i3 windows 7 operating system. The dock function (Kcal/mol) developed by the Auto dock program was used to evaluate and study the co-relation of binding affinity with % drug binding (Bioanalytical method-HPLC) and lipophilicity.

Preparation of Ligands and Nuclear Receptor for Docking: The preparation of N^{10}-substituted acridones was performed as follows,

- ✓ Conversions of 2D to 3D; sdf to pdb format.
- ✓ Correcting structures and energy minimization.
- ✓ Chemsketch for drawing, displaying and characterizing chemical structures.

Preparation of Nuclear Receptor: The structure of DNA-topoisomerase complex-I was retrieved from PDB (PDB ID- 1ej9; Organism- *Homo sapiens*).

Docking using Auto Dock 4.2 Software: The ligand library has been generated by gathering all 12 acridone analogs in Auto dock software.

The library preparation helps to make an easy comparative study between ligands by performing simultaneous docking of multiple ligands against the nuclear receptor.

Prepared ligand structures were then docked to the binding site using the default procedure implemented in Auto dock, and the binding affinity for the acridone analogs is given in **Table 1**. **Fig. 1** shows the docking pose of compound 5 to DNA (PDB ID:1ej9) with a high binding affinity of -7.01 kcal/mol.

TABLE 1: BINDNG AFFINITY OF ACRIDONES

Compounds	Binding affinity (Kcal/mol)
2	-6.53
3	-6.42
4	-5.74
5	-7.01
6	-6.41
7	-5.38
8	-6.04
9	-4.75
10	-5.93
11	-5.36
12	-6.08
13	-5.93

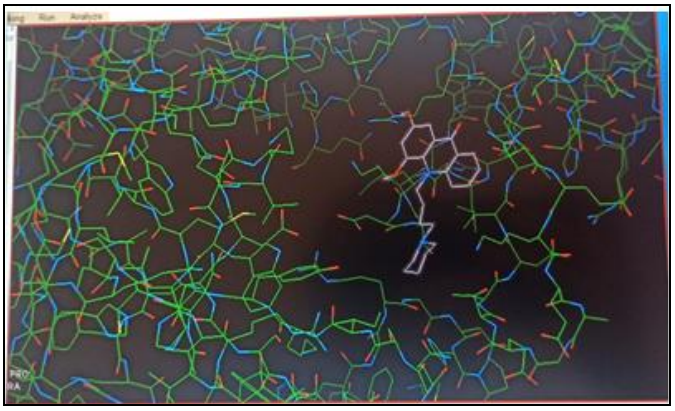


FIG. 1: DOCKING POSE OF COMPOUND 5 TO DNA (PDB ID: 1EJ9)

Bioanalytical Method (Hplc- % Drug Binding):

DNA-binding properties: The DNA-binding properties of the compounds 2-13 (salt form) were studied by HPLC method [8]. The acridone derivatives upon addition of CT-DNA showed binding affinity and the percentage binding of acridones in contact with CT-DNA is shown in **Table 2. Fig. 2** shows the HPLC chromatogram of the compound 12 binding to CT-DNA, progressive addition of DNA led to strong percentage binding in the peak area in all the compounds studied. The percentage of DNA bound to the compounds 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 were found to be 81.95, 88.77, 76.59, 66.94, 76.37, 90.26, 80.93, 87.12, 75.54, 85.99, 90.78 and 88.85 respectively. **Fig. 3** shows the representative percentage binding of the compound 12 (7, 14, 21, 28 µg/ml) in an increasing concentration of CT-DNA. The percentage binding of acridones in the presence of DNA is believed to be a result of their intercalation with the DNA. The relative binding affinities as indicated by the binding percentage were in the order of 12 > 7 > 13 > 3 > 9 > 11 > 2 > 8 > 4 > 6 > 10 > 5**.** Those with strong DNA-binding affinities 7, 12 and 13 exhibited the highest percentage of binding among the derivatives. However, the highest binding percentage was observed for compound 12 bearing planar tricyclic ring with electron-donating dimethoxy groups, linked with butyl pyrazole side chain.

TABLE 2: BINDING PERCENTAGE OF ACRIDONES IN CONTACT WITH CT-DNA

Compound	Shift in λ_{max}	% Drug binding
2	263	81.95
3	265	88.77
4	262	76.59
5	263	66.94
6	262	76.37
7	265	90.26
8	264	80.93
9	262	87.12
10	264	75.54
11	266	85.99
12	264	90.78
13	264	88.85

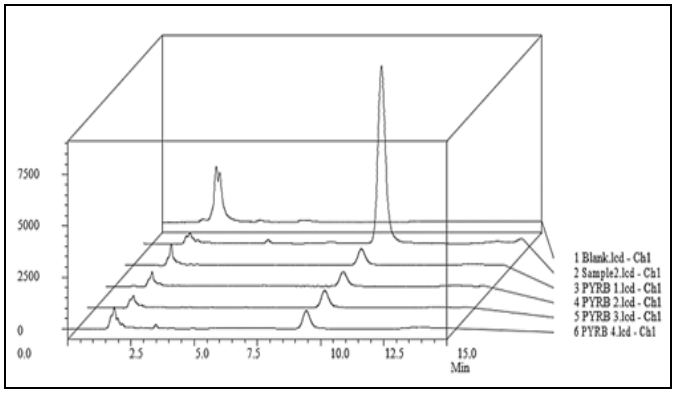


FIG. 2: HPLC CHROMATOGRAM OF COMPOUND 12 WITH CT-DNA

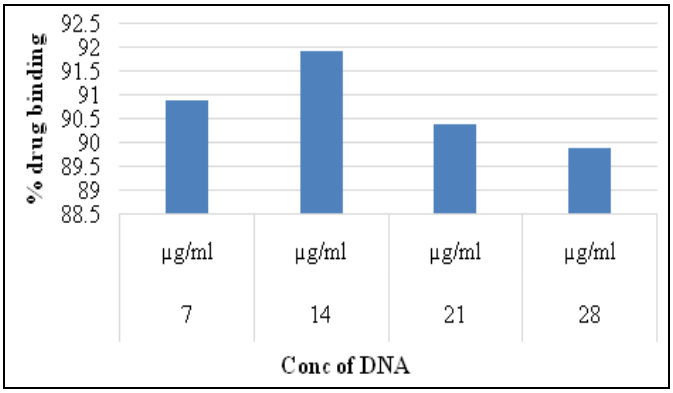


FIG. 3: % DRUG BINDING OF COMPOUND 12 WITH CT-DNA

Lipophilicity: The compounds' lipophilicity was determined using the interface of swiss drug design tools. Two computational servers did lipophilicity prediction for comparison purposes, ALOGPS and

the interface of the swiss drug design tool showed almost similar Log P values; the efficacy of a cytotoxic drug will depend in part on its ability to accumulate in cells. The efficiency of cytotoxicity mainly concentrates on the accumulation of drugs within the cells. This helps in predicting the potency of a drug. The acridone derivatives are weak bases and can exist in both charged (protonated) and uncharged (unprotonated) forms. The lipophilicity data varying from 2.84-5.09 expressed in $Log_{10}P$ are given in **Table 3**.

Substitution of hydrogen by the dimethoxy group in positions C-2, C-4 of the acridone ring, and alkyl side chain with different secondary amino groups resulted in a slight enhancement in the $Log_{10}P$ values. Additionally, it is speculated that the acridone nucleus with dimethoxy group at positions C-2, C-4 may exhibit a higher affinity for membranes or are more readily taken up into the cells than that with hydrogen atoms. Compound 8 with $Log_{10}P$ (5.09) showed a maximum binding percentage with DNA.

TABLE 3: CORRELATION OF BINDING AFFINITY AND LOG P WITH % DRUG BINDING

Compound code	Binding affinity (Kcal/mol)	Log *P*	% Drug binding
2	-6.53	3.18	81.95
3	-6.42	2.84	88.77
4	-5.74	4.01	76.59
5	-7.01	3.72	66.94
6	-6.41	3.76	76.37
7	-5.38	3.46	90.26
8	-6.04	5.09	80.93
9	-4.75	3.67	87.12
10	-5.93	3.37	75.54
11	-5.36	3.02	85.99
12	-6.08	3.33	90.78
13	-5.93	3.01	88.85

Compound 12 with a binding affinity of -6.08 kcal/mol and LogP 3.33 showed 90.78 % drug binding, and compound 7 with a binding affinity of -5.38 kcal/mol and LogP 3.46 showed 90.26% drug binding. This experimental data suggests a poor correlation between binding affinity and lipophilicity. 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.

CONCLUSION: The new acridone analogs derived from the acridone-containing secondary amino group at the terminal end of the alkyl chain have been showing their strong DNA binding ability through intercalation with CT-DNA. In particular, the effect is more pronounced with the butyl side chain compared to propyl chain. The substitution of hydrogen by $-OCH_3$ group increased their ability to bind DNA. Careful examination of the results revealed that dimethoxy butyl derivatives have higher activity than dimethoxy propyl derivatives. Further on co-relating bioanalytical results with docking results and Log P showed a reliable % drug binding profile with respect to binding affinity, on the other hand, a poor correlation in context to Log P. Concerning these observations, we concluded that this series could be developed as a promising cytotoxic agent as DNA intercalators.

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CONFLICTS OF INTEREST: Authors do not have any Conflicts of Interest

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