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IN-SILICO DOCKING AND SYNTHESIS OF NOVEL ACRIDONE DERIVATIVES FOR THEIR DNA BINDING PROPERTIES BY HPTLC AS ANTI-CANCER AGENTS

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ABSTRACT

Twelve novel compounds were designed and assessed through molecular docking, with scores ranging from -6.12 to -8.80. The top three compounds 10, 11, and 12 showed the highest Glide scores of -8.20, -8.45, and -8.80 were selected and synthesized. The Novel N^{10}-substituted acridones were synthesized via Ullmann Condensation and cyclization followed by a Phase transfer catalysis and Nucleophilic substitution reaction. The synthesized compounds were characterized by NMR and Mass spectroscopy. DNA binding study was carried out using the HPTLC method where binding affinity of a drug with DNA had been identified. The percentage of the drug bind with CT-DNA for the Compound 10, 11 and 12 was 89.08, 90.76 and 97.18 respectively. The DNA binding study indicated that the compounds 10, 11, and 12 showed good interactions with CT-DNA. Among, compound 12 exhibiting high affinity to CT-DNA with unbounded drug remained of 2.82%. These results demonstrated the effectiveness of integrating molecular docking, and DNA binding study to identify and develop promising therapeutic agents.

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INTRODUCTION

The cancer is a deviant proliferation of tissue that can metastasize. The World Health Organization (WHO) described around 9.6 million deaths globally in 2018[1]. Cancer is caused by abnormalities of genetic material in affected cells. Cancer progression is a multistep phenomenon including mutations of oncogenes and suppressor genes, interrupting the regular cell cycle. Interruptions are changes in DNA sequences, chromosomal aberrations (translocations, deletions, and amplification), and changes in chromatin structures. These changes cause aberrant DNA methylation or acetylation of histones [2]. DNA topoisomerases maintain the topology of DNA during replication, recombination, transcription, and repair of DNA [3]. Two topoisomerases: Topo I operate by cutting DNA strands whereas Topo II reduces the double strand of DNA. Two functionally different Topo II isomers were seen in mammals, Topo IIα and Topo IIβ [4,5]. The therapeutically used anti-cancer medications interfere with DNA metabolism, inhibiting nucleic acid production or hindering DNA breakage and repair mechanisms [6,7].

The acridone has a chemical structure containing two benzene rings fused with a ketone group and nitrogen atom at 9^{th} and 10^{th} position, respectively, which provides a planer structure. This chemical scaffold is reported to have biological activities such as anti-cancer, anti-malarial, anti-viral, and modulation of multi-drug resistance (MDR) [8]. The Compounds, (Fig.1) based on the tricyclic template were researched for better antitumor agents and reviewed. Now many acridone compounds such as aconitine, imidazoacridone (C-1311), and, Triazoleacridone (C-1305) are in clinical trials [9].

Fig.1: 1. Aconitine, 2.Imidazoacridone (C-1311), 3.Triazoleacridone (C-1305).

Literature suggests that the acridone moiety, when modified with a pentyl chain and attached to a secondary amine, significantly enhances the biological activity of the compounds. The HPTLC method for CT-DNA binding analysis is a new contribution to the field, offering a novel technique that had not been previously documented. In this study, molecular docking study was performed on N^{10}-substituted acridones to evaluate their binding affinity. Three of the docked compounds showed the highest binding affinity and were subsequently synthesized. To investigate their interaction with CT-DNA, a novel HPTLC method was developed, marking a new approach for the analysis of DNA binding property.

EXPERIMENTAL

Molecular Docking

The molecular docking, a computational method plays a more significant role in drug discovery and development by revealing the most usable site of the bio- macromolecules, like DNA, that is used to bind small molecules called DBL (DNA binding ligands). Docking studies were performed to analyze the mode of binding between DBL and DNA.

Molecular docking is considered the "key and lock" hypothesis used to find the best-fit orientation of ligands and proteins. The three-dimensional crystal structure of MADS-box/MEF2D N-terminal domain bound to dsDNA and HDAC9 deacetylase binding motif (PDB ID:8Q9R) downloaded from the RCSB Protein data bank (PDB) and water molecules from the receptor were removed followed by Kollaman's charges added then polar hydrogens were added using AutoDock 1.5.6 tools. The structures of ligands were drawn using ChemDraw 16.0 and copied as SMILES. The Avogadro tool was used to generate the PDB file of ligands with energy minimization. All structures were saved as pdb file format and converted into .pdbqt to carry out the docking studies in AutoDock 1.5.6 tools. The grid file was generated and saved as a .gpf file. The Lamarckian Genetic Algorithm (LGA) was used to find the conformers with the lowest binding energies and the .dpf file was generated to dock. The final docking task was performed by using AutoDock 1.5.6. After completion of docking, receptor-ligand interactions were analyzed. The highest binding energy conformation of ligand was selected and interactions were visualized in the Biovia discovery studio client 2021 [10].

Chemicals and Reagents

Chemicals and reagents used in synthesis were AR grade and procured from local suppliers. ECX-JEOL 400(S) AVIII400(L) was used to record NMR spectra in $CDCl_3$ and reported in Hertz. Shimadzu LC solution was used to record mass spectra in ES+ ionization mode.

I. Synthesis of 2-(phenylamino)benzoic acid / 2-(1-amino, 4-methyl, phenyl) benzoic acid / 5-methyl-2-(1-amino, 4-methyl, phenyl) benzoic acid:

5g of anhydrous potassium carbonate was added slowly to the mixture of 0.032mol (5 g) of o-chlorobenzoic acid/ 2-bromo-5-methyl benzoic acid, 0.032mol (4.4g) of Aniline/ Toluidine, and 0.2g copper powder in 30 ml isoamyl alcohol, and the contents were allowed to reflux for 8 hours. Isoamyl alcohol was removed by distillation, and the mixture was poured into 1 L of hot water and acidified with concentrated hydrochloric acid. The precipitate was filtered, washed with hot water, and collected. The crude acid was dissolved in an aqueous sodium hydroxide solution, boiled in activated charcoal, and filtered. On acidification of the filtrate with concentrated hydrochloric acid, the precipitate was obtained, washed with hot water, and recrystallized using methanol to obtain a brownish solid. The reaction was monitored by TLC using Petroleum ether: Ethyl acetate (5:5) as a mobile phase, and the spots were observed under UV.

II. Synthesis of Acridone/ 2-methyl Acridone/2,7 dimethyl Acridone

5g of 2-(phenylamino)benzoic acid / 2-(1-amino, 4-methyl, phenyl) benzoic acid / 5-methyl-2-(1-amino, 4-methyl, phenyl) benzoic acid was taken in a flask to which 50 g of polyphosphoric acid was added. Agitated and heated in a water bath at 100 °C for 4 hours. The appearance of a yellow color indicated the completion of the reaction. The contents were poured into 1 L of hot water and made alkaline using liquor ammonia, and the light yellowish precipitate formed was filtered, washed with hot water, and collected. The compound, Acridone / 2-methylacridone / 2,7-dimethylacridone was recrystallized from glacial acetic acid. The progress of the reaction was monitored by TLC using Petroleum ether: Ethyl acetate (5:5) as the mobile phase, and the spots were observed under UV.

Acridone: M.P-335, λ_{max}-366nm, Rf- 0.31
2-methylacridone: M.P-320, λ_{max}-355nm,Rf- 0.33
2,7-dimethylacridone: M.P-325, λ_{max}-345nm, Rf- 0.36

III. Synthesis of N^{10} - alkylated Acridone/ 2-methyl Acridone/2,7-dimethyl Acridone via Phase Transfer Catalysis

1g (0.0028 mol) of Acridone/2-methyl Acridone/2,7-dimethyl Acridone was dissolved in 25 ml tetrahydrofuran, 17ml of 6N potassium hydroxide and 0.43 g (0.0013 mol) of tetrabutylammonium bromide (TBAB) were added, and the reaction mixture was stirred at room temperature for 30 min and added 1-bromo-5-chloropentane (0.013 mol) slowly into the reaction mixture and stirred for 24 hours at room temperature. Tetrahydrofuran was evaporated, and the aqueous layer was extracted with chloroform. The chloroform layer was washed with water, and the organic layer was dried over anhydrous sodium sulphate and evaporated using a Rota evaporator to obtain the intermediate.

IV. Secondary amine substitution for 10-(5-chloropentyl)Acridin-9(10H)-one/ 10-(5-chloropentyl)-2-methyl Acridin-9(10H)-one/10-(5-chloropentyl)-2,7-dimethyl Acridin-9(10H)-one

N^{10} - alkylated Acridone/ 2-methyl Acridone/2,7-dimethyl Acridone was dissolved in 60 ml of anhydrous acetonitrile and 1.68g potassium iodide and 3.3g of potassium carbonate were mixed and refluxed for 30 min. and 0.89ml (0.009mol) of isatoic anhydride was added slowly and refluxed for 15 hours until a substantial amount of the product was formed as confirmed 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 sulphate, and evaporated to obtain the product.

1-(5-(9-oxoacridin-10(9H)-yl)pentyl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione

MP-300<.λ_{max}:248.Rf: 0.69. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, 1H), 8.30 (dd, 1H), 8.22(s, 1H), 7.86 (d, 1H), 7.79 (d, 1H), 7.63-7.50 (m, 2H), 7.41 (t, 3H), 7.17 (t, 1H), 3.6 (t, 2H), 2.4(s, 3H), 1.88 (p,2H), 1.38(p,2H), 0.89(p,2H). **^{13}C NMR (100 MHz)** δ 178.35, 167.77, 150.82, 141.28, 139.48, 134.61, 133.62, 132.69, 132.12, 130.42, 127.64, 126.51, 125.56, 125.14, 122.04, 120.97, 120.61, 117.84, 77.25, 58.79, 54.85, 29.69, 25.91, 23.96, 22.93, 21.01.**Mass m/z** = 426.61 (M+H)

1-(5-(2-methyl-9-oxoacridin-10(9H)-yl)pentyl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione

MP-300<. λ_{max} :248.Rf: 0.69. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, 1H), 8.30-8.25(s, 3H), 8.12-8.05(dt, 2H), 7.86(dt, 1H), 7.62-7.52 (m,2H), 7.47 (dd, 2H), 4.30-4.25(m,2H), 1.91(p, 2H), 1.69(p,2H), 0.88(p, 2H). **^{13}C NMR (100 MHz):**δ 177.65, 160.56, 148.74, 141.52, 139.72, 132.69, 131.95, 130.71, 128.43, 127.34, 124.97, 122.07, 121.95, 114.87, 114.02, 51.28, 45.26, 27.34, 27.04, 25.24, 20.94. **Mass m/z** = 440.77 (M+H)

1-(5-(2,7-dimethyl-9-oxoacridin-10(9H)-yl)pentyl)-2H-benzo[d][1,3]oxazine-2,4(1H)-dione

MP-300<. λ_{max}:248. Rf: 0.69. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81(dd,2H), 8.51(dd,2H), 8.49(s,1H),7.70-7.765(t, 2H), 7.34-7.28(m, 5H), 2.60(t, 2H), 2.5(t,2H), 1.81(p,2H), 1.30(p,2H), 0.90(p,2H). **^{13}C NMR (100 MHz):**178.13, 160.56, 148.74, 141.93, 141.52, 134.60, 131.95, 128.43, 127.17, 124.97, 123.65, 122.01, 117.15, 116.66, 114.87, 51.28, 45.26, 27.34, 27.04, 25.24. **Mass m/z** = 454.25 (M+H)

DNA-binding study by HPTLC

Deoxyribonucleic acid (DNA), an essential biological macromolecule, consists of two polynucleotide strands coiled to form a double helix. The region where the two strands are close to each other is called the minor groove, while the other region where they

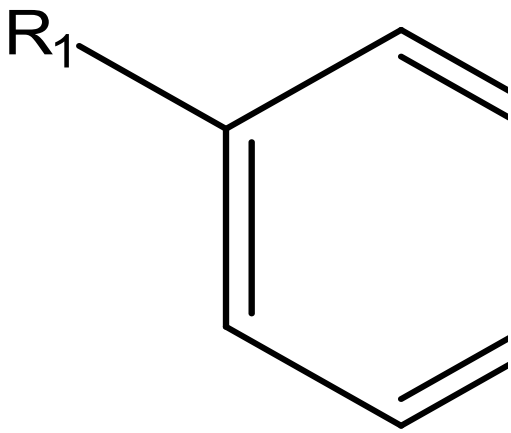
are away is called the major groove. DNA plays a crucial physiological part in the life process. However, when small molecules bind to the DNA it alters or inhibits the normal function of DNA. Studies have proved that small molecules bind to DNA mainly through non-covalent interactions. Planar heterocyclic molecules such as acridones act as intercalators. Acridones stack between adjacent DNA base pairs, resulting in significant p-electron overlap, thus interfering with hydrogen bonds between nucleotide base pairs [11].

The solutions of CT-DNA in sodium phosphate buffer (pH 7.2) with different concentrations (0 to 120µm) were used. Binding was performed at fixed concentrations of drugs (10µm) in a sodium phosphate buffer (PH7.4). Small aliquots of concentrated CT-DNA (3.3mM) were added into the solution at concentrations from 0 to 120 µm and stirred for 5 minutes. The DNA binding affinity of the compounds was performed using salinized (Reversed-Phase: RP) silica gel 60F254 (0.25 mm) plates (Merck). The TLC plates were pre-developed with a methanol/50 mM sodium hydrogen phosphate (pH 7.4) mixture (5:5v/v).

The prepared dilutions of CT-DNA and fixed drug concentration mixtures were applied to the HPTLC plate using a microliter syringe attached to an Automatic TLC Sampler (CAMAGLinomat5) with 2-mm band size at a speed of 150nL/s. Before that, testing compounds were incubated with CT-DNA (50 mM sodium hydrogen phosphate, pH 7.4) for 30 minutes at 37 °C.

The plates were developed with a methanol/50 mM sodium hydrogen phosphate (pH 7.4) mixture (5:5v/v). The loaded TLC plate was developed in a 20 × 10 cm^2 pre-saturated glass chamber under controlled temperature (25 °C ± 2 °C) and humidity(6%±5%).

SCHEME-1:



Substitute

Table:1. Binding score of Novel N^{10} substituted acridones.

Compound	Figure code	Glide score
	01	-6.12
	02	-6.53
	03	-6.91
	04	-7.00
	05	-7.21
	06	-7.70

	07	**-7.30**
	08	**-7.60**
	09	**-7.90**
	10	**-8.20**
	11	**-8.45**

	12	**-8.80**

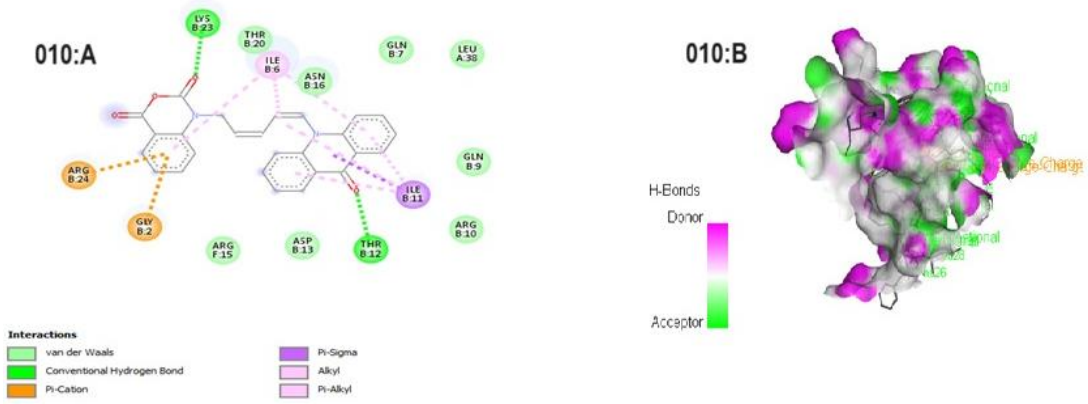


Fig.2: 3D and 2D binding pose of compound (10) with 8Q9R.

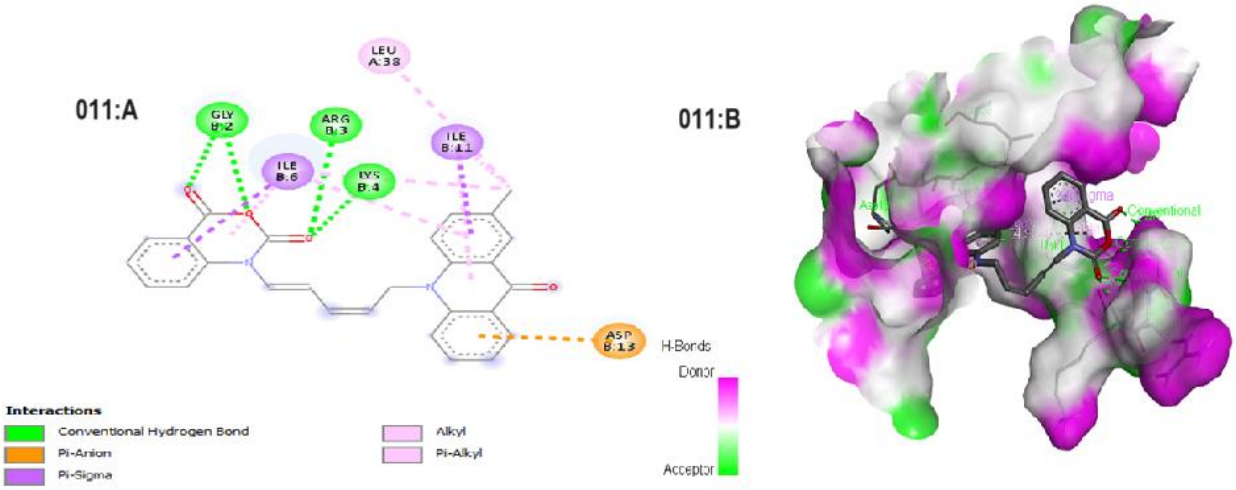


Fig.3: 3D and 2D binding pose of compound (11) with 8Q9R.

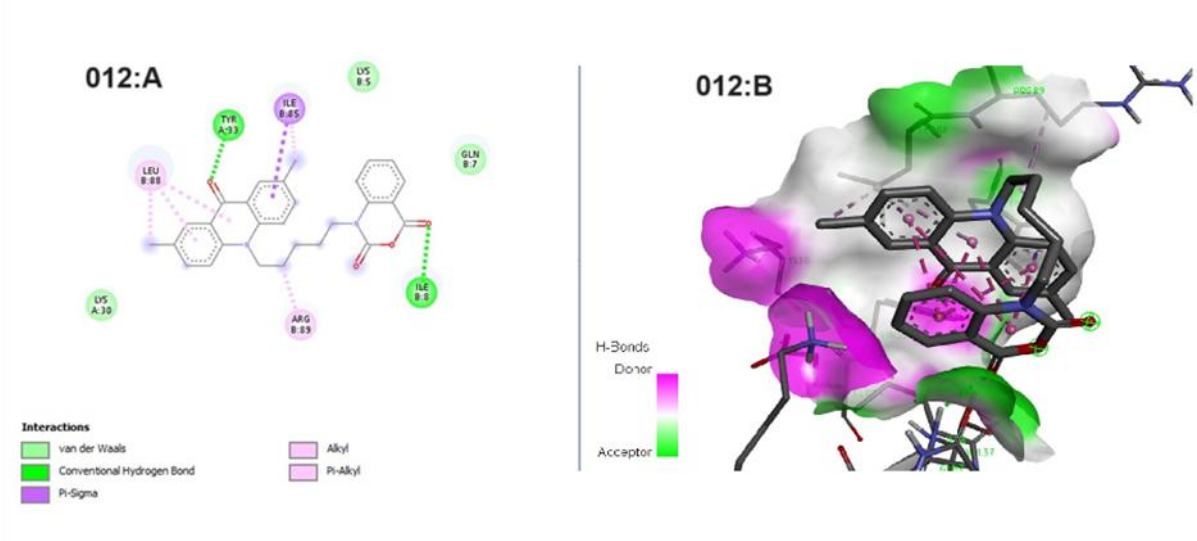


Fig.4: 3D and 2D binding pose of compound (12) with 8Q9R.

Table-2: Area of the peaks for Compound 10.

Peak	DNA Concentrations(in µm)	Rf	Area of the peak
1	0	0.69	72.06%
2	20	0.69	54.89%
3	40	0.69	43.92%
4	60	0.69	31.82%
5	80	0.69	21.91%
6	100	0.69	15.53%
7	120	0.69	10.92%

Table-3: Area of the peaks for Compound 11.

Peak	DNA Concentrations(in µm)	Rf	Area of the peak
1	0	0.69	67.87%
2	20	0.69	41.25%
3	40	0.69	34.71%
4	60	0.69	29.42%
5	80	0.69	21.77%
6	100	0.69	17.58%
7	120	0.69	9.24%

Table-4: Area of the peaks for Compound 12.

Peak	DNA Concentrations(in µm)	Rf	Area of the peak
1	0	0.69	69.68%
2	20	0.69	55.79%
3	40	0.69	43.93%
4	60	0.69	31.15%
5	80	0.69	23.32%
6	100	0.69	16.41%
7	120	0.69	2.82%

RESULTS AND DISCUSSION

Molecular Docking

Molecular docking studies of 12 compounds ranging from **-6.12**to **-8.80** were carried out in AUTODOCK 1.5.2. Version. The protein was obtained from the RCSB protein data bank **(PDB ID: 8Q9R)** for MADS-box/MEF2D N-terminal domain bound to dsDNA and HDAC9 deacetylase binding motif, docking results of these compounds are given in **Table no. 1**.The compound **12** shows better intercalation between the nucleotide base pairs with glide score **-8.80.** From docked pose of compound **12(fig.4)**, the acridone ring with methyl-substituted formed pi-sigma and conventional hydrogen bond interaction with nucleotide base pairs (B:85, B:88, A:33). Along, the alkyl chain (B:89) and oxygen atom on isatonic anhydride interacted conventional hydrogen bond (B:8).The compounds **10, 11,** and **12** showed the**-8.20, -8.45,** and **-8.80** glide scores respectively were synthesized using the given synthetic **Scheme-1**. Protein-ligand interaction of the synthesized compounds is shown in the following figures, **10 (fig.2), 11 (fig.3), and 12 (fig.4)**.

Chemistry

The first step involved Ullmann condensation of o-chloro benzoic acid/o-bromo 5-methyl benzoic acid with Aniline/Toluidine. Cyclization was performed using polyphosphoric acid. N^{10} -alkylation with alkyl halides was difficult due to the weakly basic nature of nitrogen of the acridone nucleus. However, it was achieved with a phase transfer catalyst (PTC) for a better yield. Substituted acridone was stirred at room temperature with 1-Bromo 5-Chloro pentane in a two-phase system consisting of an organic solvent (tetrahydrofuran) and an aqueous potassium hydroxide solution in the presence of tetrabutylammonium bromide. This led to the formation of the respective N-alkylated products with good yield. Further, ammonium salt transported hydroxide ions from the aqueous phase to the organic phase, where the actual reaction occurred. The results were interpreted by deprotonation of the acridones by OH^-, transferred by the catalyst into the organic layer. The anion formed as a phenolate-stabilized anion, which subsequently undergoes alkylation to form an aromatized system. Iodide catalyzed nucleophilic substitution of the N^{10} substituted acridones with the Isatonic anhydride was carried out by refluxing for different time intervals in the presence of potassium carbonate in anhydrous acetonitrile giving the free bases.

DNA Binding studies by HPTLC

The DNA-binding property of compounds **10, 11,** and **12** were studied by HPTLC method. The binding percentage of acridones in the presence of DNA is attributed to their ability to intercalate with the DNA. The concentration of non-bound drugs (free drugs) was observed in this method by the intensities of the spot. The decreasing spot intensities suggested enhanced drug binding to DNA. The relative binding affinities, as indicated by the binding percentages, showed that as the concentration of CT-DNA increased, the drug concentration decreased, which was evident in the TLC. The compounds **10, 11,** and **12** showed good binding interaction with CT-DNA. The binding order of the compound follows **10 < 11 <12**. The compound **12** bearing electron-donating dimethyl groups on the tricyclic nucleus, linked with the pentyl-isatonic anhydride side chain has the highest binding with CT- DNA compared to the compound **10** (Plane substituted acridone) and compound **11(**2-methyl substituted acridone). The Percentage area of compounds **10, 11,** and **12** are shown in **Tables 2, 3,** and **4** respectively.

This shows a significant effect of an electron-donating group on the Acridone scaffold compared to the unsubstituted Acridone moiety.

CONCLUSION

The correlation between molecular docking and DNA binding study suggest that compound 12 with electron-donating groups (CH_3) at positions 2 and 7 on the acridone ring has a significant increase in biological activity. Furthermore, the presence of a pentyl chain improves lipophilicity, whereas isotonic anhydride increases compound solubility. These structural modifications work together to maximize the therapeutic potential of the synthesized compounds. Compound 12 in particular showed the highest binding affinity towards CT-DNA, indicating its potential as a lead candidate for future development. Overall, the study emphasizes the significance of rational drug design and the structure-activity relationship (SAR) in developing effective therapeutic agents.

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