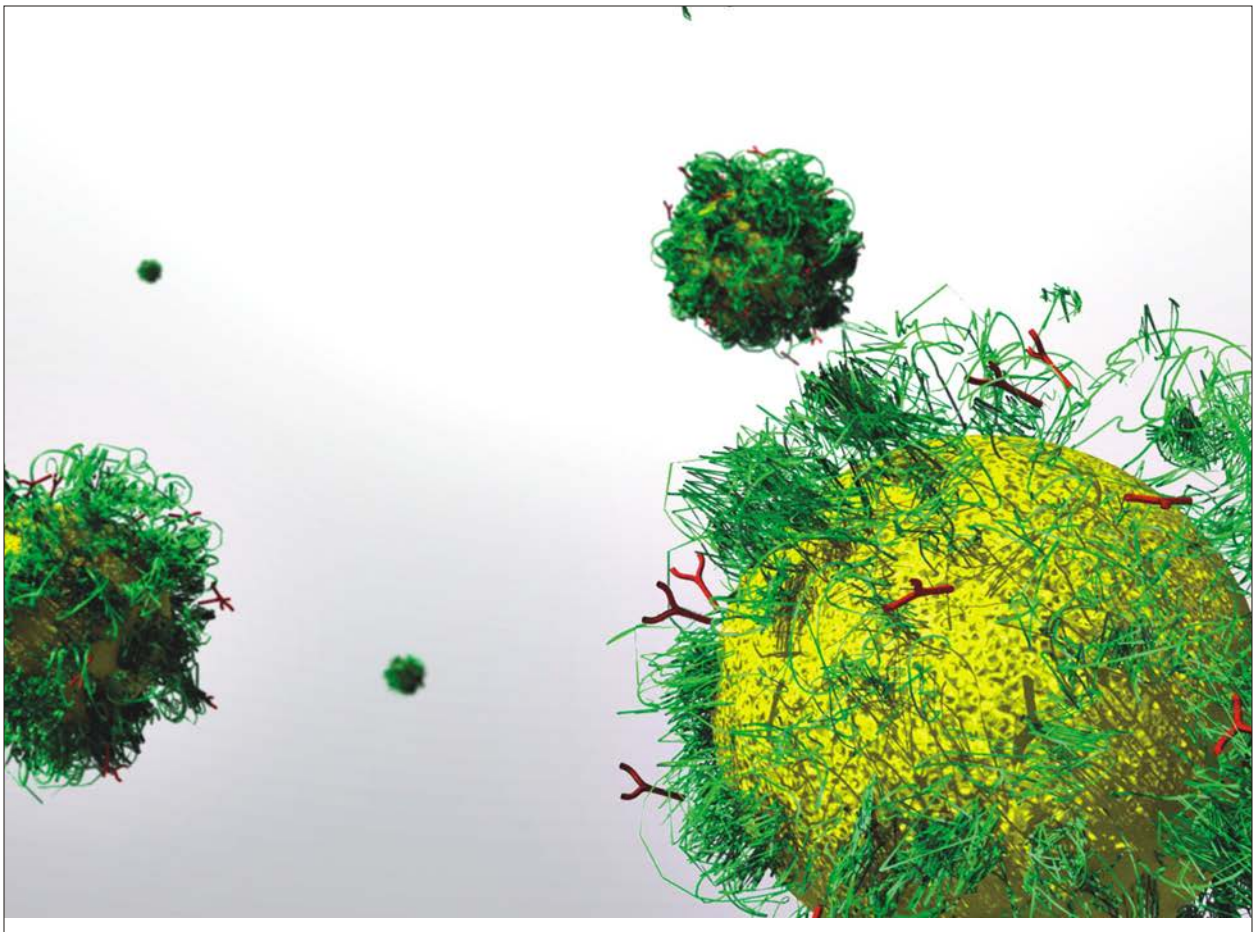

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In silico and *in vivo* Study the Mode of Action and Effect of Some Biurets as DNA Binding Agent

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ABSTRACT

In silico biology, including computers, databases, methods and algorithms are used for statistically analyzing the data to achieve the extract information and to identify the relationships between these data sets. This study aimed to provide alternative and new drugs that connect to DNA by using *in vivo*, *in silico* and find the best option from the newly designed series of drugs. Out of 143 DNA dodecamer crystal structures available in PDB, we have selected three structures and retrieved from the Protein Data Bank (PDB), with minor/major groove or both groove biding mode. Eighteen experimental ligands were docked using AutoDock 4.3 program into the active sites of selected drug-DNA structures. We docked our experimental ligands into three modeled DNA structured and compared those to internal evaluated ligands (drug compounds include ditercalinium, adriamycin and propamidine) and observed the same binding sites in experimental ligands in comparison to internal ligands. Docking results of 6m and 6p compounds into oligonucleotide in comparison to adrimycin and propamidine showed almost same binding fashion. Our results display that the most plausible mode of action of these experimental drugs as DNA binding agents is through intercalation of AT base pairs-linker chain and azole-minor groove. Accordingly, other DNA-drug crystal structures can be used as good patterns for further improvements using *in silico* and structure-based drug design methods.

Key words: Molecular docking, DNA binding, *in silico*, *in vivo*, AutoDock, biuret

INTRODUCTION

Bioinformatics tools and *in silico* biology, including computers, databases, methods and algorithms that are used for statistically analyzing the data, which its goals are, help to reach to the extract information and to identify the relationships between these data sets (Rahim, 2010; Rahim, 2008a, b).

DNA is one of the supreme imperative targets of numerous chemotherapeutic agents. Considering the mode of interaction, it is possible to conveniently categorize chemotherapeutic drugs targeting DNA into two major classes (Yang and Wang, 1999). These classes include covalent and non-covalent binding; comprise interactive and minor-or major-groove binding, respectively.

Biuret is a group of chemical compound that includes two molecules of urea, which is soluble in hot water (Fouladdel *et al*., 2010). Moreover, these chemical compounds also describes a group of organic drugs with the functional group-(HN-CO-)2N-. Previously a research showed that biurets have higher cytotoxicity and lower cell viability in a concentration and time-dependent fashion, when is used against human breast cancer T47D cell line (Fouladdel *et al*., 2010). Biurets is reported that could have potential as pharmacological tools against parasitic protozoan such as the visceral leishmaniasis (Adibpour *et al*., 2012). Because of the higher cost and lower digestibility,biuret sare less preferred than urea, hence, such specifications also justifies its minor risk of ammonia toxicity (Fonnesbeck *et al*., 1975; Oltjen *et al*., 1969).

The purpose of this study was to provide alternative and new drugs that connect to DNA by using *in vivo, in silico* and bioinformatics tools and find the best option from the newly designed series of drugs.

MATERIALS AND METHODS

Nucleotide molecules (DNA) preparation: Crystal structures of three different DNA-drug complexes include ditercalinium (PDB ID: 1D32) (Nunn and Neidle, 1995), adriamycin (PDB ID: 1D12) (Frederick *et al*., 1990), propamidine (PDB ID: 102D) (Gao *et al*., 1991) and those spatial coordinates were studied using the Protein Data Bank (PDB) (Kirchmair *et al*., 2008). Then, the water molecules and the ligand were removed from each PDB file using UCSF Chimera software (Pettersen *et al*., 2004) and text editor software. Furthermore, hydrogen and partial atomic charges (AMBMER) were added using HyperChem 8.0 and non-polar hydrogens were inserted to their carbon atoms using AutoDock tools as well.

Ligand molecules preparation: The DNA molecules were removed from each PDB file using UCSF Chimera software and TextPad software to prepare the ligand molecules. Herein, non-polar hydrogens were added and then a short minimization were performed and Gasteiger-Maesili method (Gilson *et al*., 2003), used to calculate partial atomic charge using AutoDock tools and HyperChem 8.0 based on the parameter explained in previous study (Sobhani *et al*., 2006).

Biurets molecules preparation: All 18 biurets molecules listed in Table 1 were admitted to internal evaluation by the same procedure as described above. Furthermore, for all biurets molecules the unrotatable bonds were managed using AutoDock tools.

Internal validation: The prepared DNA and ligand molecules were docked using AutoDock tools and Vina AutoDock (Fig. 1) and their simulation results were obtained (Table 2).

Virtual screening: All deigned biurets containing eighteen 3D-structures of molecules was screened virtually on one of the developed DNA by PyRx. After the virtual screening we selected compounds for further testing by two different methods using visual inspection and automatic filtering. In summary, we selected compounds 6m and 6p with best and significant DNA binding activities.

Docking protocol: After selection, DNA target preparation wizard of FlexX has been used to prepare target DNA. Target DNA was subjected to energy minimization using the steepest descent technique to eliminate bad contacts between DNA atoms using the GROMOS 96 implementation

Table 1: Structures, IC_{50} and percent of cell survival of biurets 6a-r

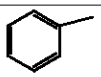
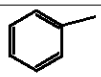
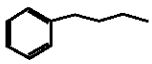
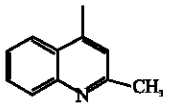
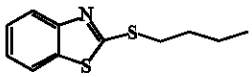
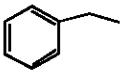
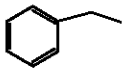
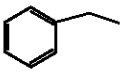
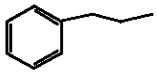
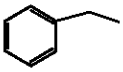
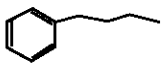
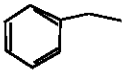
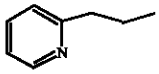
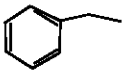
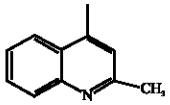
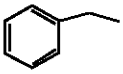
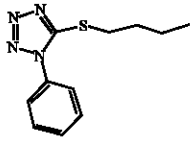
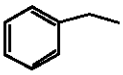
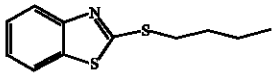
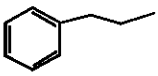
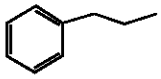
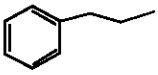
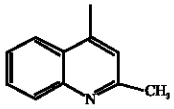
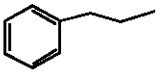
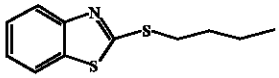
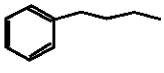
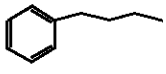
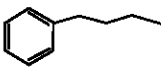
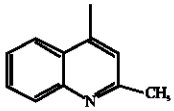
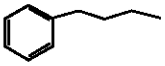
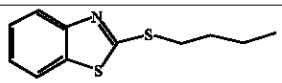
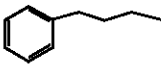
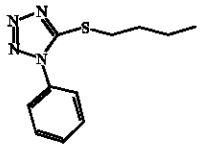
Compound name	Compound structure R	Compound structure R'	[a]IC_{50} (µM)	[b]Survival(%)
6a (1)			60	82.35
6b (2)			50	75.23
6c (3)			35	67.25
6d (4)			35	64.1
6e (5)			75	91.3
6f (6)			55	75.97
6g (7)			70	88.5
6h (8)			75	79.22
6i (9)			25	49.8
6j (10)			50	73.62
6k (11)			25	54.32
6l (12)			50	78.76
6m (13)			10	28.15
6n (14)			45	79.82
6o (15)			70	84.53
6p (16)			20	45.72

Table 1: Continue

Compound name	Compound structure R	Compound structure R'	[a]IC_{50} (µM)	[b]Survival (%)
6q (17)			60	74.92
6r (18)			55	67.86

[a]IC_{50}: Compounds was determined at 2 days exposure using MTT assay, [b]Percent survival of T47D cells following exposure to 25 µM concentration of compounds was determined after 2 days exposure using MTT assay

Table 2: Docking results of internal validation and experimental ligands and those corresponding pIC_{50} values

	102D		1D12		1D32			
Compound[ref]	FDE	ΔG	FDE	ΔG	FDE	ΔG	pIC_{50}	ΔG_{obs}
Internal evaluation								
Propmidine	-18.84	-9.37	-	-	-	-	-	-
Adrimycin	-	-	-13.48	-9.8	-	-	-	-
Ditercalinium	-	-	-	-	-21.06	-17.36	-	-
Experimental ligand evaluation								
6a (1)	-7.01	-6.41	-5.76	-3.05	-6.46	-4.25	4.22	0.035545
6b (2)	-7.59	-6.99	-7.57	-6.97	-9.37	-8.77	4.30	0.029621
6c (3)	-8.58	-7.69	-8.64	-7.75	-10.39	-9.50	4.46	0.020735
6d (4)	-8.66	-7.77	-8.39	-7.47	-9.45	-8.56	4.46	0.020735
6e (5)	-7.05	-6.45	-7.36	-6.76	-8.42	-7.82	4.12	0.044432
6f (6)	-7.56	-6.96	-7.45	-6.85	-9.03	-8.43	4.26	0.032583
6g (7)	-7.39	-6.76	-7.77	-7.17	-9.08	-8.48	4.15	0.04147
6h (8)	-7.33	-6.73	-7.18	-6.58	-8.76	-8.16	4.12	0.044432
6i (9)	-7.46	-6.57	-8.09	-7.20	-9.67	-8.78	4.60	0.014811
6j (10)	-8.46	-7.57	-8.21	-7.32	-10.72	-9.83*	4.30	0.029621
6k (11)	-9.02	-8.13	-8.63	-7.74	-9.48	-8.59	4.60	0.014811
6l (12)	-7.35	-6.57	-7.36	-6.76	-9.05	-8.45	4.30	0.029621
6m (13)	-7.89	-7.0	-8.47	-7.58	-10.17	-9.28	5.00	0.005924
6n (14)	-8.69	-7.80	-8.38	-7.49	-9.24	-8.35	4.35	0.026659
6o (15)	-7.69	-7.09	-7.58	-6.98	-9.31	-8.71	4.15	0.04147
6p (16)	-8.03	-7.14	-8.57	-7.68	-10.28	-9.39	4.70	0.011848
6q (17)	-9.12	-8.23	-8.34	-7.45	-9.37	-8.48	4.22	0.035545
6r (18)	-9.29	-8.40	-8.79	-7.90	-10.48	-9.59	4.26	0.032583

in the Swiss-Pdbviewer program suite (Kaplan and Littlejohn, 2001). The ChemSketch (ACDLABS 12.0) tool was used to draw the structure of the all experimental compounds and then the structures converted to 3D structure with the help of optimization tool. The prepared DNA and ligand molecules were docked using FlexX (Kramer *et al.*, 1999), as a part of LeadIT 2.0.1 (www.biosolveit.de). The default parameters of FlexX were used for each docking run. To test the reliability and reproducibility of the docking protocol, the obtained DNA and ligand structures were docked first. The grid maps of all five DNA molecules were calculated individually using AutoGrid

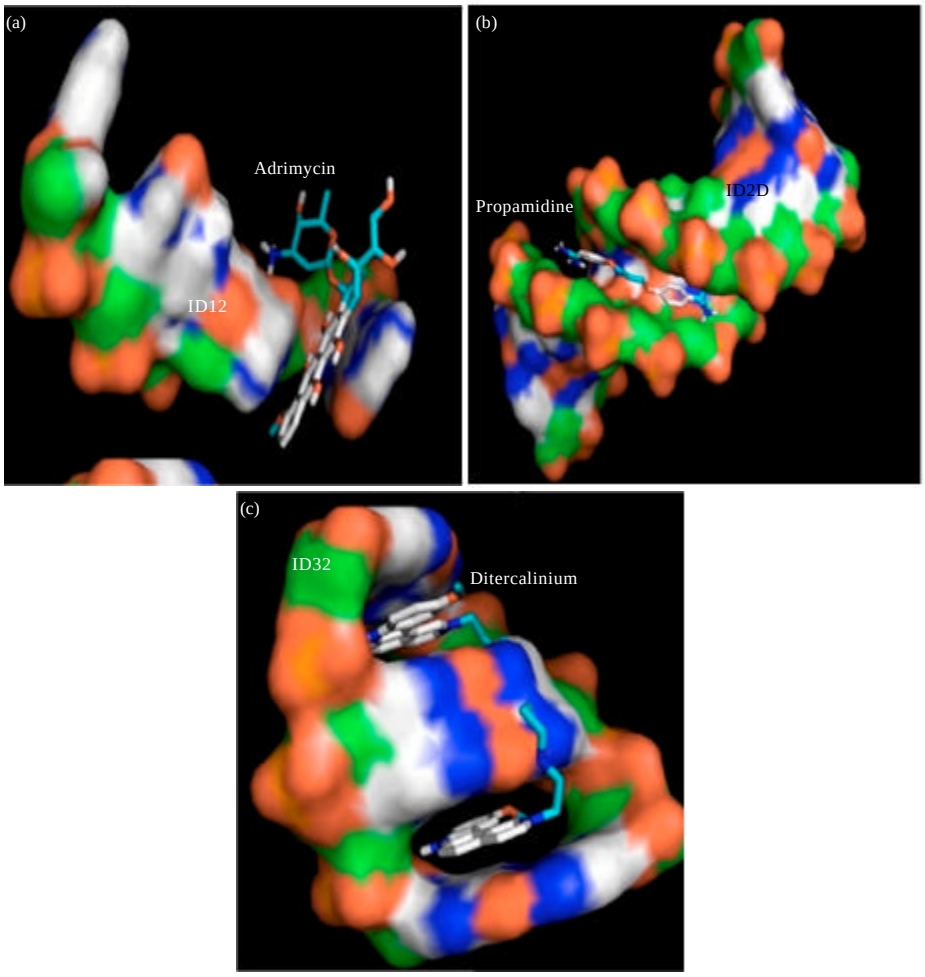


Fig. 1(a-c): Internal validation using 3 different DNA structures (colored area is the solvent-excluded surface (SEA) and related drug molecules (stick structure), Docking of (a) Adrimycin into oligonucleotide (PDB ID: 1D12), (b) Propamidine into oligonucleotide (PDB ID: 102D), (c) Ditercalinium into oligonucleotide (PDB ID: 1D32). All the images were created using PayMol molecular graphic software

part of AutoDock tools focusing on sufficient large to include active site and significant part of surface as well. The grid's points were 65×65×65 for 1D32 and 1D12, 70×70×70 for 102D (grid spacing of 0.375 Å).

However, according to the known location of original ligand in the complex that was retrieved from the PDB, the cubic grids were centered on the ligand's binding site. Automated docking was performed using AutoDock 4.2 with Lamarckian Genetic Algorithm (LGA) to model DNA-biurets interaction and binding, in which 100 multiple, independent docking runs were carried out to increase the performance of docking programs. Finally, cluster analysis was carried out on the observed docking values base on the root mean square (RMS, 0.5Å). The program LigPlot was used to calculate the binding interactions (Wallace *et al.*, 1995).

Statistical analysis: The obtained data were analyzed using SPSS 17.0. The correlation between two sets of rankings was calculated using Spearman correlation coefficient. The p-values less than 0.05 were considered as significant.

RESULTS

Out of 143 DNA crystal structures available in PDB, we have selected five DNA-ligand complex structures include ditercalinium (PDB ID: 1D32), adriamycin (PDB ID: 1D12), propamidine (PDB ID: 102D) and those spatial coordinates were studied using the Protein Data Bank (PDB), with minor/major groove or both groove biding mode. We have used the vina-AutoDock, to predict the biding affinity of internal validation and experimental ligands to all DNA molecules. The structures, IC_{50} and percent of cell survival of 18 experimental ligands have been described in detail (Table 1). Figure 1 illustrated the interaction of the above drugs and DNA molecules. The docking results of experimental ligands to all DNA molecules, resulting FDE and ΔG values for each model and those related experimental pIC_{50} and ΔG_{obs} are presented in Table 2. The drugs that used in internal evaluation include ditercalinium, adriamycin and propamidine were docked into those corresponding DNA molecules and the related FDE and ΔG values were calculated (Table 2).

We used quantitative and qualitative considerations of AutoDock given complexes to select most probable conformation. First we choose the conformations with the lowest binding affinity as the starting points, then the chosen conformations were screened and those with lowest FDE and best pIC_{50} were narrowed the selection. Finally, those selected conformations were subjected to qualitative analysis based on the location or orientation of ligand in the DNA structures. This procedure was repeated till we found the best conformation. Then, we docked those selected experimental ligands (10, 17, 18) into three modeled DNA structured and compared those to internal evaluated ligands (drug compounds include ditercalinium, adriamycin and propamidine). We observed the same binding sites in experimental ligands in comparison to internal ligands (Fig. 2). Furthermore, we superimposed the experimental compounds to internal ligands and compared the localization into the DNA binding sites. Docking results of compounds 17 (Fig. 3a, b) and 18 (Fig. 3c, d) into oligonucleotide in comparison to adrimycin showed almost same binding fashion. Besides, docking results of 17 (Fig. 4a, b) and 18 (Fig. 4a, b) compounds into oligonucleotide in comparison to propamidine also showed almost same binding fashion, while docking results of 17 (Fig. 5a, b) and 18 (Fig. 5a, b) compounds into oligonucleotide showed almost same binding fashion but in opposite direction in comparison to ditercalinium.

Docking procedures basically aim to identify the correct conformation and to predict the affinity of ligands in the binding pocket of the target DNA/protein. In this study all biurets compounds docked with target DNA using FlexX. For validating the software, the DNA was re-docked with the already bound ligand. The highest absolute value of interactions energies were considered be the best and proper ligands. In that, all the ligand poses had the good FlexX score and energy compared to propamidine. There was a significant correlation between FDE and ΔG observed values with experimental ΔG for two DNA binding models include 1D12 and 1D32 (Table 3). This finding noticeably validates that the observed FDE value by AutoDock may consider as a good tool to allow rapid evaluation of DNA-ligand interactions.

Evidently among those selected DNA binding models, bisintercalation DNA model (1D32) showed a better correlation between binding energy and $\Delta G_{(obs)}$ ($R = 0.66$, $p = 0.002$), $\Delta G_{(obs)}$ and $\Delta G_{(exp)}$ ($R = 0.66$, $p = 0.002$), followed by groove binding plus intercalation model (1D12) (Fig. 6). Hence, the other selected model (102D) showed no significant difference (Table 3).

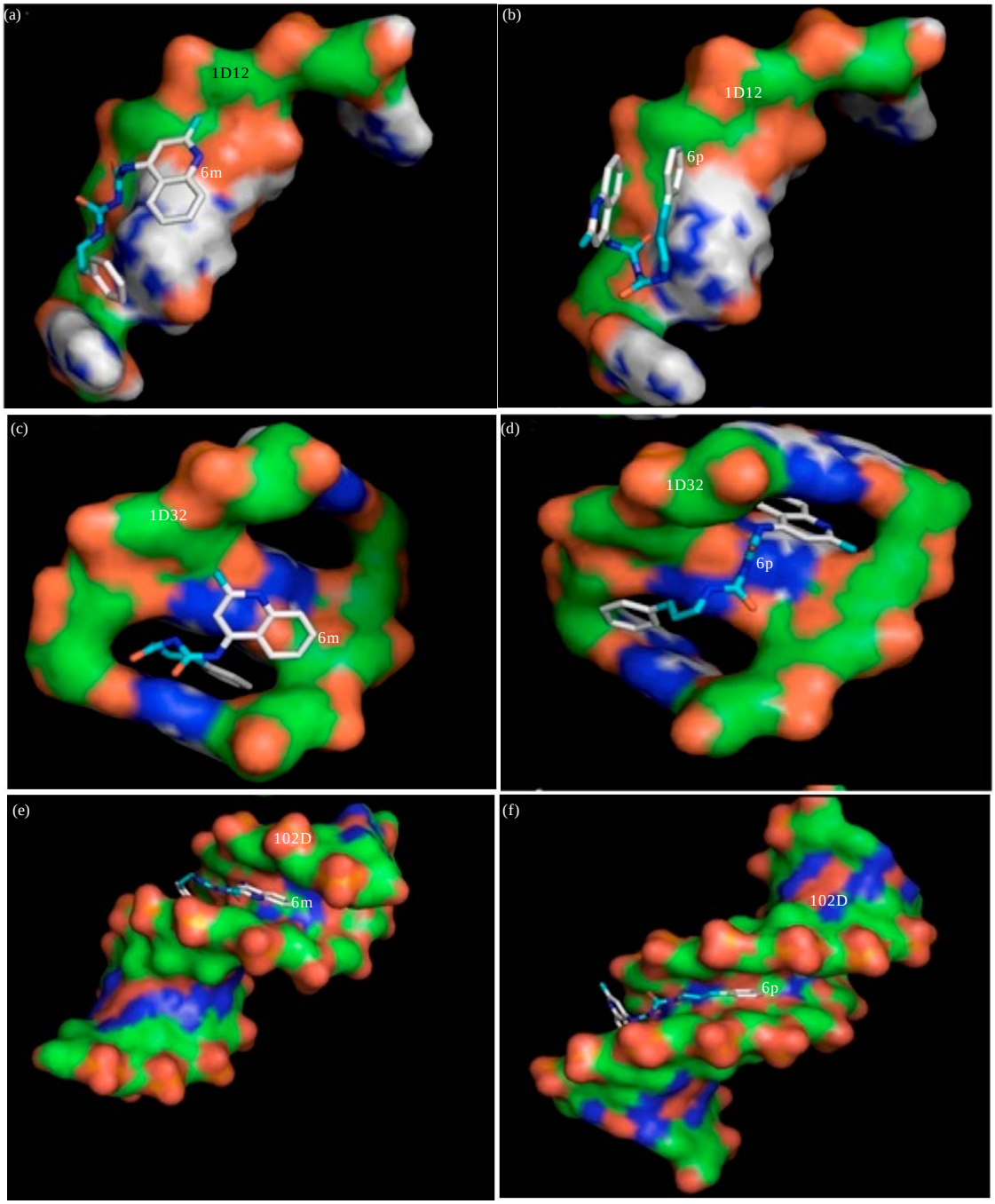


Fig. 2(a-f): Experimental ligand validation using 3 different DNA structures (colored areais the Solvent-excluded surface (SEA) and related drug molecules (stick structure), Docking of 6 m and 6 p compounds into oligonucleotide (a, b) (PDB ID: 1D12), (c, d) (PDB ID: 1D32) and (e, f) (PDB ID: 102D), all the images were created using PayMol molecular graphic software

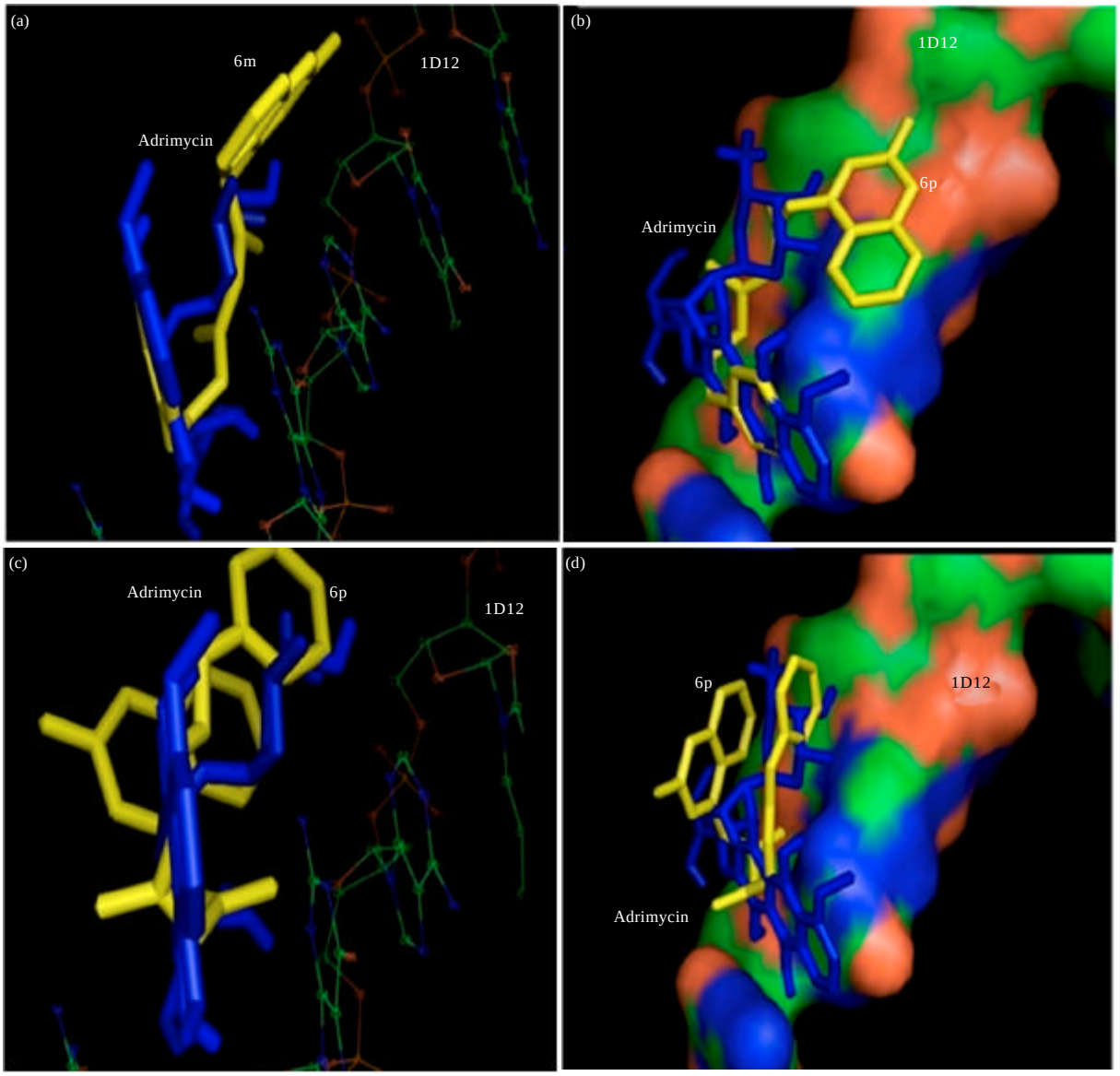


Fig. 3(a-d): Internal and experimental ligand binding comparison using DNA structures (PDB ID: 1D12, colored area is the solvent-excluded surface (SEA)) and related drug molecules (stick structure), Docking of (a, b) 6m (yellow) and (c, d) 6p (yellow) compound into oligonucleotide in comparison to adrimycin (blue), all the images were created using PayMol molecular graphic software

Table 3: Correlation coefficients between calculated and experimental binding energies for the three different DNA binding models

	1D12[A]		1D32[B]		102D[C]	
Spearman correlation coefficient	R	p-value	R	p-value	R	p-value
FDE with $\Delta G_{(obs)}$	0.596	0.11	0.667	0.002	1.64	0.120
$\Delta G_{(Exp)}$ with $\Delta G_{(obs)}$	0.596	0.11	0.565	0.014	1.06	0.304

A: Groove binding plus intercalation DNA model, B: Bisintercalation DNA model, C: Groove binding DNA model

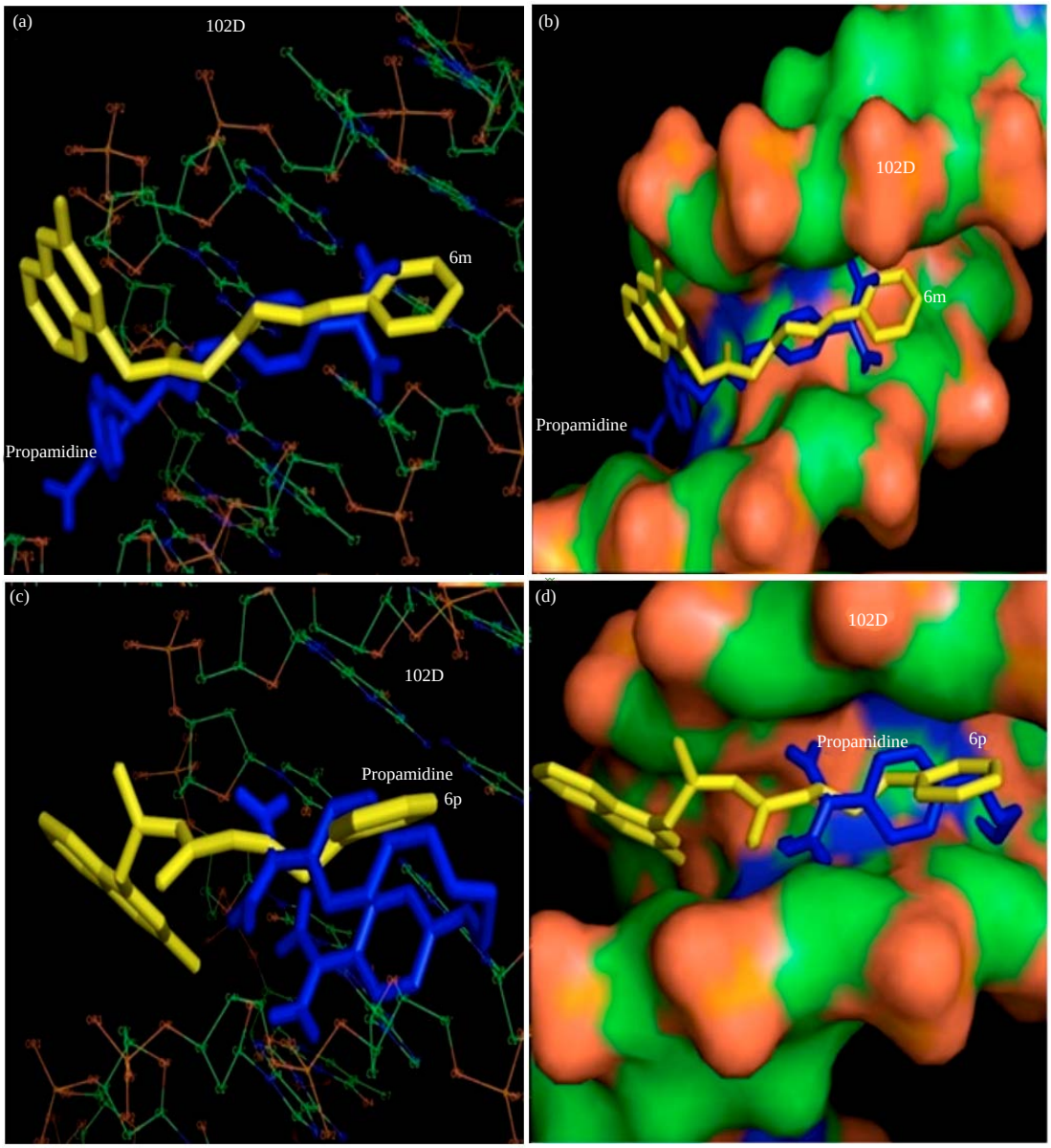


Fig. 4(a-d): Internal and experimental ligand binding comparison using DNA structures (PDB ID: 102D, colored area is the solvent-excluded surface (SEA) and related drug molecules (stick structure), Docking of (a, b) 6m (yellow) and (c, d) 6p (yellow) compound into oilgonucleotide in comparison to propamidine (blue), all the images were created using PayMol molecular graphic software

DISCUSSION

Docking results using three different DNA binding models revealed that, in spite of variations in experimental ligands in the context of functional groups and minor variation in inter molecular interaction, the binding mode is very similar. We used the 18 compound with the highest binding affinity and the best pIC_{50} of all experimental ligands have been selected for further evaluation and

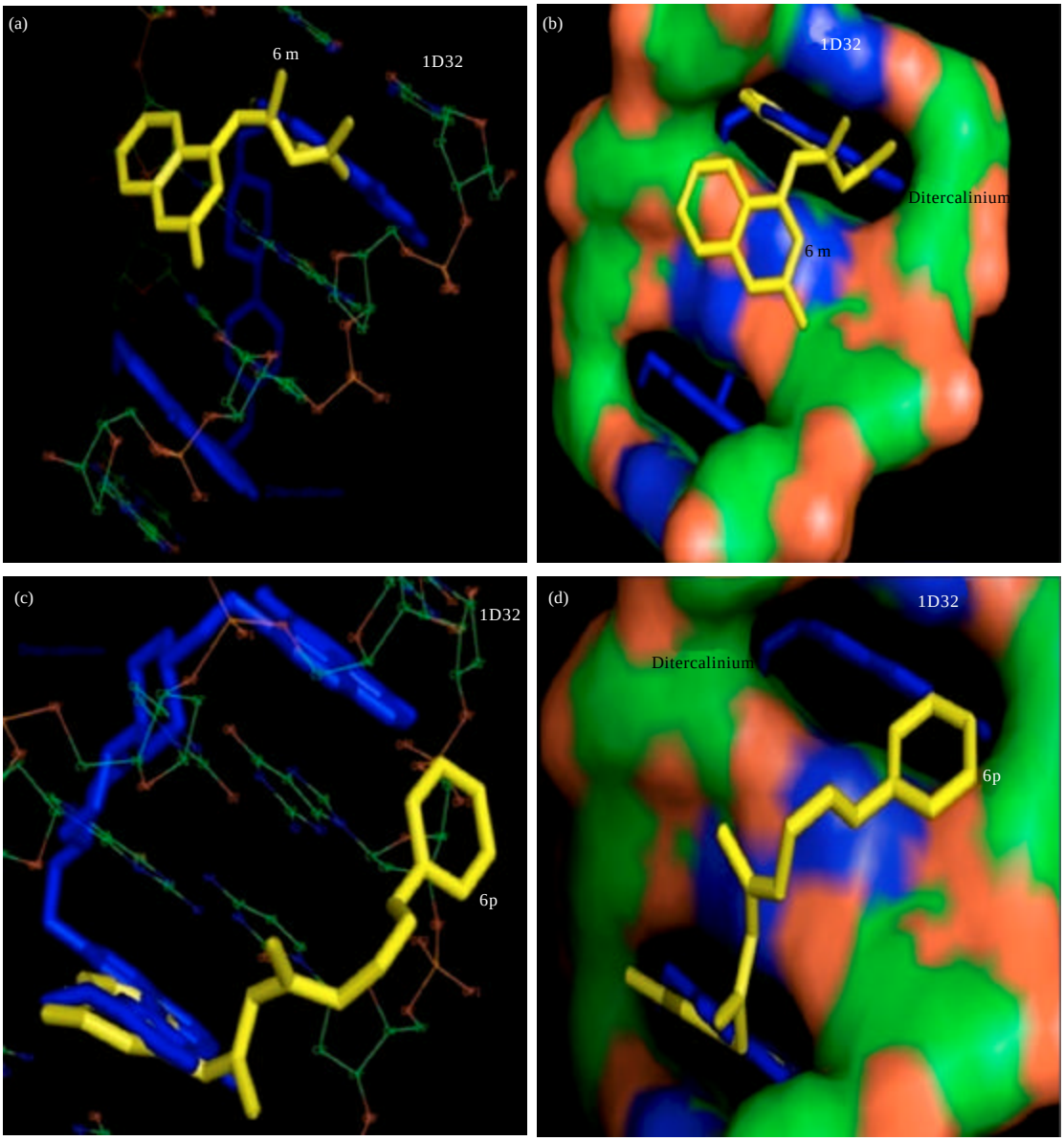


Fig. 5(a-d): Internal and experimental ligand binding comparison using DNA structures (PDB ID: 1D32, colored area is the solvent-excluded surface (SEA) and related drug molecules (stick structure), Docking of (a, b) 6m (yellow) and (c, d) compound into oligonucleotide in comparison to ditercalinium (blue), all the images were created using PayMol molecular graphic software

discussion of DNA binding models. From the results obtained, it will be essential to understand the important structural features required enhancing the inhibitory activities and further it will help to produce augmented inhibitory compounds.

Although, among those three DNA binding models, groove binding model (102D) shows a better correlation (R = 1.64, p = 0.12), but bisintercalation DNA model (1D32) had a significant correlation (R = 0.66, p = 0.002) (Fig. 6). Docking simulations study of all three binding models showed that

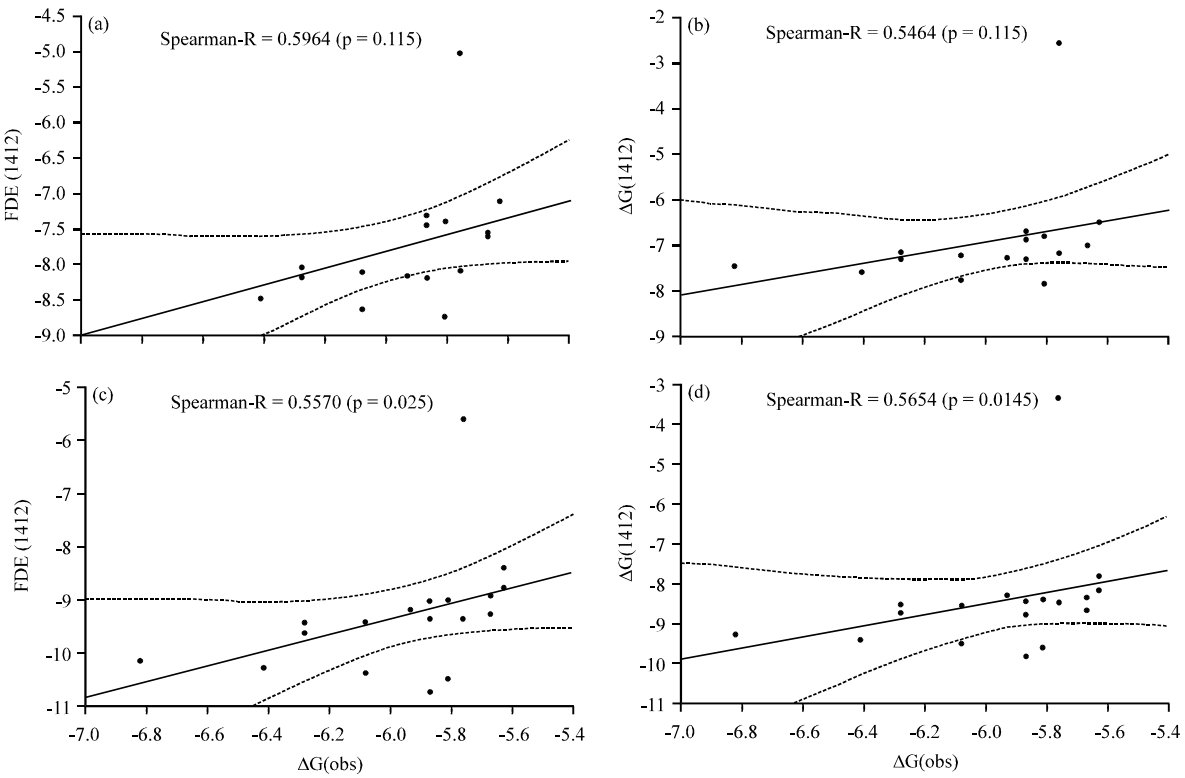


Fig. 6(a-d): Theoretically predicted final dock energy (FDE: kcaL moL^{-1}) resulted from groove binding plus intercalation model vs. experimentally measured ones for biurets used in this study, (a) and (b) shows groove binding plus intercalation DNA model (1D12), (c) and (d) shows Bisintercalation DNA model (1D32)

even with dissimilarities in terms of functional groups, the majority bind in a very similar fashion with minor variation in intermolecular interactions within each model. Consequently, we used those compounds with the nearest pIC50 value to the average of all experimental ligands and highest FDE for arguing intermolecular interactions within each binding model.

Docking study of the 102D binding model showed that, three selected compounds extend well in the GT-rich sites, while the azole ring and the linker chain forming simple hydrophobic interactions to the extended minor groove (Fig. 7a). Further stabilization of this complex is related to three hydrogen bonds between three azole ring nitrogen molecules and two adenine and thymine bases. Besides, in the 1D12 model, the compounds of interest extend into AT-richsites, forming stacking interactions (Fig. 7b). Further stabilization of this complex is related to four hydrogen bonds between three azole ring nitrogen molecules and thymine bases. While, in the bisintercalation model (1D32), a p-stacking interactions formed by the azole ring with two consecutive AT base pairs at the two ends of DNA molecules (Fig. 7c). There are three stabilizing hydrogen bonds formed between two nitrogens of azole ring and nearby guanine.

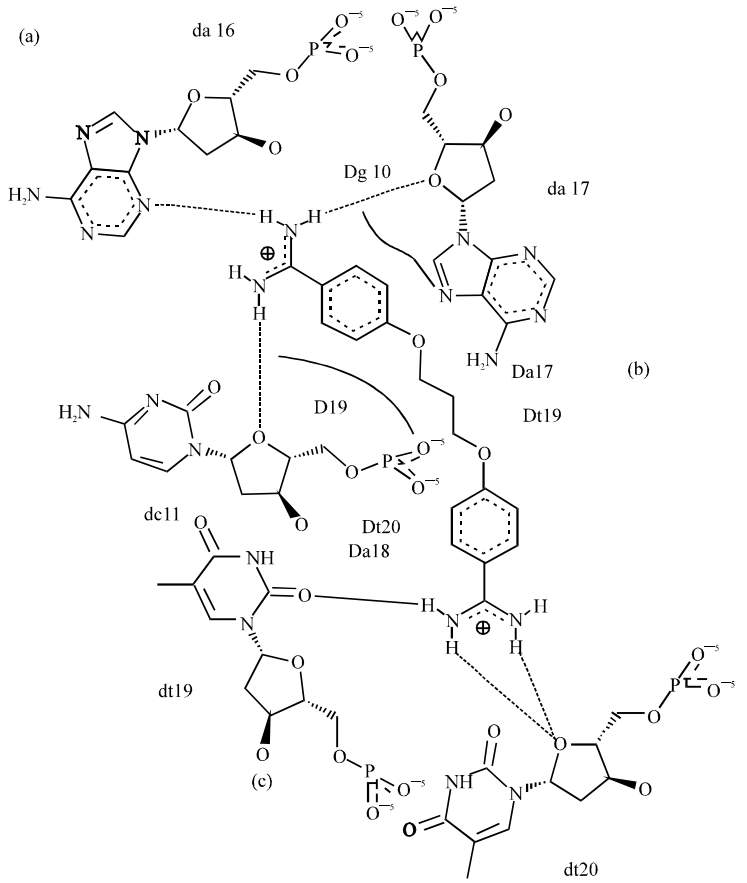


Fig. 7(a-c): Two-dimensional representations of the binding interactions between the selected compounds and nucleotide residues of DNA molecules (a) Groove binding DNA model (102D), (b) Groove binding plus intercalation model (1D12), (c) Bisintercalation model (1D32)

CONCLUSION

Although, several deficiencies exist in molecular docking techniques when dealing with DNA molecules as targets, but may reveal biologically sound results in case of the difficult drugs targets reaction. Here we attempt to present a novel approach that can be useful to other research for DNA-ligand interactions. Our findings show that the most plausible mode of action of biurets is through intercalation of azole ring and linker chain with two consecutive AT base pairs in the minor groove. This similarity suggests that, other DNA- drug crystal structures may act as worthy templates for further researches in the field of the structure-based drug design using *in silico* methods. The authors report no declarations of interest.

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