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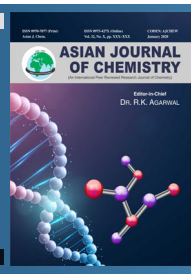

Cyanuric Chloride Containing Chalcones for Possible Breast Cancer Treatment: Synthesis, Antimicrobial and *in silico* Screening

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In this work, we have synthesized efficient antibacterial compounds with anticancer novel molecules based on cyanuric chloride containing chalcone moiety. For this, novel triazine-based organic molecules were synthesized by using cyanuric chloride and 2,4-dichloro-1-ene(4-hydroxyphenyl)phenone and characterized by elemental analysis, FT-IR, NMR and UV-visible spectrometry techniques. Melting point of the molecules were increased with an increase in substitution on cyanuric chloride. The minimum inhibitory concentration (MIC) value of the synthesized compounds showed an excellent result on Gram-negative bacteria with low MIC value of 1.95 µg/mL. Gram-positive bacteria showed little resistance to the synthesized drug. The synthesized compounds were tested for their use as an anticancer drug using *in silico* screening method. The synthesized compounds *in silico* molecular docking method using breast cancer protein (BRCA2) confirms that triazine derivative with all three chlorine molecules replaced by 2,4-dichloro-1-ene(4-hydroxyphenyl)phenone showed highest binding energy with the value of -9.1900 Kcal/mol which is in agreement with the observed high MIC value obtained for Gram-negative bacteria. The synthesized molecules preferentially targeted the topoisomerase II of the bacteria. Overall, an efficient antimicrobial drug is synthesized using a simple preparation method.

Keywords: Drug design, Structure activity relationship, Chalcone, Antimicrobial activity.

INTRODUCTION

Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) finds enormous applications in dye chemistry, agriculture and textile industry. Cyanuric chloride is used to synthesize a variety of organic compounds [1] due to the ease of simple replacement of chlorine. Triazine-based chalcone finds important role in heterocyclic chemistry mainly due to their good biological activities [2]. Several derivatives of triazines show antibacterial [3], antimicrobial [4], herbicidal [5], antimalarial [6], anti-cancer [7], antiprotozoals [8] and antitubercular activities [9,10]. The chlorine atom in cyanuric chloride can be easily replaceable which is facilitated by the ring N-atom. Wagle and Venkataraman [11] prepared alkyl compounds from alcohol and acyl chlorides from carboxylic acids, in both cases they used cyanuric chloride as a reagent. Baldaniya and Patel [10] synthesized some novel triazines as a biological potent agent. Similarly, Patel *et al*. [12] synthesized several derivatives of cyanuric chlorides and tested for their antimicrobial activity.

In general, reliability and the accuracy of any biological studies can be evaluated using molecular docking and modeling studies which can also provide the possible interactions of molecules with that of target receptors. Identifying drug targets *via* bioinformatics tools is done by *in silico* methods. *in silico* Approach has great advantage due to the fact it drastically reduces the cost which is involved in the drug designing. It also reduces the time span which needed for a drug to pass through the drug discovery pathway. Due to these two reasons, *in silico* methods finds an important place in the novel drug discovery [13,14].

With these backgrounds, we have focused to develop an efficient antibacterial active chalcone compound containing triazine as a base structure. The synthesized compounds are subjected to molecular docking against DNA gyrase as the target protein for an antibacterial agent. The targeted molecules were synthesized using 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) and 2,4-dichloro-1-one(4-hydroxyphenyl)phenone.

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EXPERIMENTAL

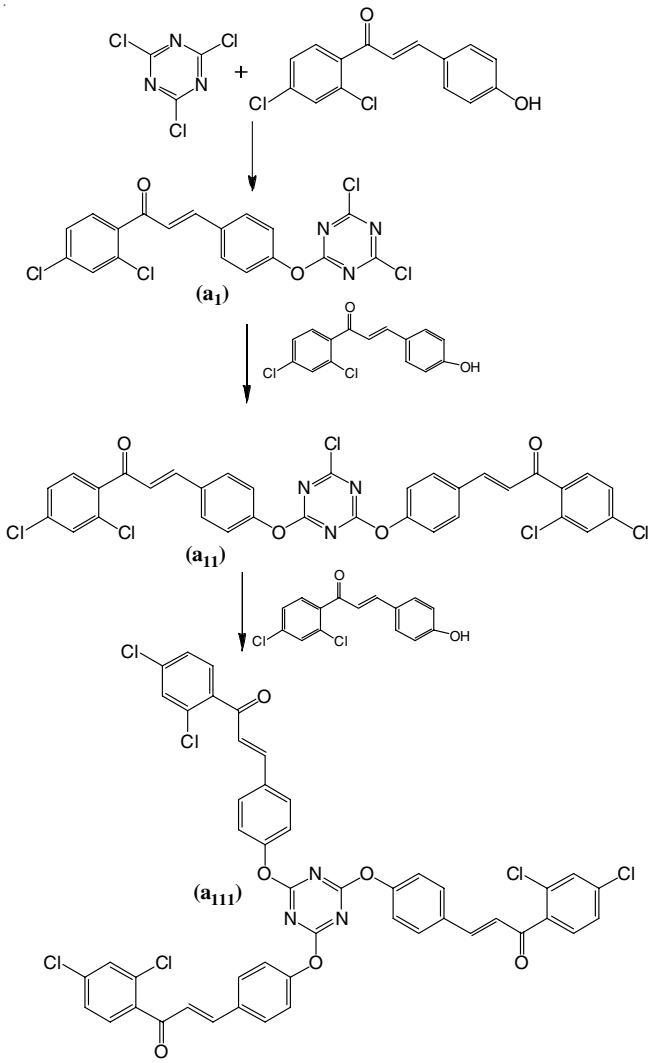
2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride) is used as received from Aldrich Chemicals. Chalcone (2,4-dichloro-1-one(4-hydroxyphenyl)phenone) was synthesized according to reported method [15]. IR spectrum is recorded using ALPHA Bruker FT-IR spectrophotometer by KBr pellet method. Bruker FT-NMR spectrophotometer is used to measure ^{1}H NMR spectra using $CDCl_3$ as a solvent and TMS as an internal reference. UV response of the compounds is recorded using model UV 320 instrument which is supplied by LABINDIA in tetrahydrofuran (THF) solvent. Elemental analysis was done using Colemen C-H-N analyzer. MTCC stains bacteria *viz. Escherichia coli* (MTCC 739) and *Staphylococcus aureus* (MTCC 3381) were supplied by Christian Medical College (CMC), Vellore, India.

General procedure for the replacement of chlorine from cyanuric chloride: In general, first, secone and third chlorine atoms from cyanuric chloride can be replaced at 0-5, 30-50 and 60-80 ºC, respectively. General procedure for the synthesis of cyanuric chloride derivatives is followed. In an round-bottom flask containing organic compound (containing hydroxyl or amine as a functional group) in acetone and acetone containing cyanuric chloride is added with constant stirring for 6 h. During the reaction, HCl would be evolved and neutralized using 10 % Na_2CO_3 solution. The desired product can be obtained by pouring the reaction contents into crushed ice. The obtained solid was filtered and crystallized using ethyl alcohol (**Scheme-I**). All the synthesized compounds were found to be freely soluble in high polar solvents such as DMF, DMSO, acetone, chloroform, alcohol, tetrahydrofuran and insoluble in hexane and benzene.

Synthesis of (*E*)-3-(4-((4,6-Dichloro-1,3,5-triazine-2yl)oxy)phenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (a1): Cyanuric chloride (0.01mol, 1.84 g) and 2,4-dichloro-1-one(4-hydroxyphenyl)phenone (0.01 mol, 2.93 g) was dissolved in acetone were used for the synthesis of compound **a1**. Yield: 86 %; m.p. 90-92 ºC, m.w. 441.1. Elemental analysis of $C_{18}H_9N_3O_2Cl_4$ calcd, (found) %: C, 49.01 (48.09); H, 2.06 (2.02); Cl, 32.06 (31.09). IR (KBr, ν_{max}, cm^{-1}): 1643 (C=O), 1597 (CH=CH-), 1338 (C-N), 811 (triazine, C-N) and 786 (Cl-C). ^{1}H NMR (δ, ppm): 7.15-7.80 (m, 7H, Ar-H), 6.7-6.9 (d, 2H, -CO-CH=CH) and 4.0 (b, OH). UV (nm): 243 (arom. CH=CH) and 330 (CH=CH).

Synthesis of 3,3-(((6-chloro-1,3,5-triazine-2,4-diyl)*bis*(oxy))*bis*(4,1-phenylene)*bis*(1-(2,4-dichlorophenyl)prop-2-en-1-one (a11): Compound **a1** (0.01 mol, 4.41 g) and 2,4-dichloro-1-one(4-hydroxyphenyl)phenone (0.01 mol, 2.93 g) were used for the synthesis of compound **a11**. Yield: 82 %; m.p. 106-107 ºC, m.w. 697.0. Elemental analysis of $C_{33}H_{18}N_3O_4Cl_5$ calcd. (found) %: C, 56.80 (56.50); H, 2.60 (2.55); Cl, 25.40 (25.30). IR (KBr, ν_{max}, cm^{-1}): 1645 (C=O), 1598 (CH=CH-), 1340 (C-N), 815 (triazine, C-N) and 770 (Cl-C). ^{1}H NMR (δ, ppm): 7.0-7.9 (m, 14H, Ar-H), 6.7-6.8 (d, 4H, -CO-CH=CH) and 4.1 (b, OH). UV (nm): 241 (arom. (CH=CH)) and 332 (vinylic (CH=CH)).

Synthesis of (1,3,5-triazine-2,4,6-triyl)*tris*(oxy)benzene-4,1-diyl)*tris*(1-(2,4-dichlorophenyl)prop-2-en-1-one (a111): Compound **a111** (0.01 mol, 6.97g) and and 2,4-dichloro-1-one(4-hydroxyphenyl)phenone (0.01 mol, 2.93 g) were used for the synthesis of compound **a111**. Yield: 85 %; m.p. 128-130 ºC, m.w. 954.1. Elemental analysis of $C_{48}H_{27}N_3O_6Cl_6$ calcd. (found) %: C, 60.40 (60.30); H, 2.85 (2.81); Cl, 22.29 (22.23). IR (KBr, ν_{max}, cm^{-1}): 1647 (C=O), 1597 (CH=CH-), 1340 (C-N), 814 (triazine, C-N) and 786 (Cl-C). ^{1}H NMR (δ, ppm): 7.0-7.8 (m, 21H, Ar-H), 6.8-6.9 (d, 6H, -CO-CH=CH) and 4.1 (b, OH). UV(nm): 245 (arom. CH=CH) and 333 (CH=CH).



Scheme-I: Synthesis of **a1**, **a11** and **a111** compounds

Minimum inhibitory concentration (MIC): The antibacterial activities of the synthesized compounds are tested against *Staphylococcous aureus* (MTCC 3381) and *Escherichia coli* (MTCC 739). By measuring turbidity, the MIC is evaluated. The experiments were repeated for three times and the average value is reported.

Statistical analysis: Unpaired Student′s t-test is used for statistical analysis and the differences were considered significant for the *p* values of < 0.01.

Protein: Using RCSB-PDB, complex 3D structures of protein and nuclic acids can be evaluated. It will provide the complete sequence of the structure and function of any protein. The DNA structure of gyrase protein (PDB: 1KZN) is obtained from the protein data bank (http://www.pdb.org). The gyrase protein is comes under topoisomerase II type. The Schrodinger protein preparation wizard is used for the preparation of the structure of protein, which contains hydrogen atoms and the

structure is further minimized and optimized using OPLS force field.

Docking methods: The protein crystal structures under investigation are obtained from the PDB. Auto-docking tools along with the graphical user interface is used to generate the grids, to calculate the dock in score and to evaluate the active conformer which can bind to the targeted protein at their active sites. The software, Auto Dock 4.1 which is based on Lamarckian genetic algorithm is used for the energy calculations. This is based on the inter-molecular interaction between the protein and the molecule. The molecule under investigation rotates in all directions to find out the exact docking position with minimal energy using known parameters provided by the Lamarckian genetic algorithm, pseudo-Solis and Wets methods [16-19]. The desktop computer (Pentium Dual core, 2 GB RAM and Windows XP operating system) is used for designing complex structures, which use software such as Pymol version 1.1, Chimera version 1.10.1 and Pose view.

Docking analysis: The binding affinity and the binding sites were evaluated in this study using two dimensions docking structure of the synthesized compounds produced by Lig Plot 1.4.5 software [20]. The docking analysis focused mainly on two factors, namely, hydrophobic interaction and the hydrogen bond between the gyrase protein with that of the groups presented in the synthesized molecules. The synthesized compounds binding sites were compared with the standard compounds to evaluate the efficiency of the synthesized compounds to act as an effective antimicrobial/anticancer drug.

RESULTS AND DISCUSSION

A chalcone having 2,4-dichloro-1-one(4-hydroxyphenyl)-phenone was synthesized according to the reported procedure [15]. The compounds **a1**, **a11** and **a111** are synthesized according to **Scheme-I**. The structures of the synthesized molecules are confirmed by spectral techniques such as, IR, ^{1}H NMR and UV-visible. FT-IR spectrum of compound **a1** shows the incorporation of chalcone moiety into the cyanuric chloride, which is confirmed by the presence of absorption of the peak at 1680-1660 cm^{-1} (Fig. 1). The NMR spectrum of compound **a1** (Fig. 2) showed the expected peak for the successful incorporation of chalcone moiety into cyanuric chloride. The presence of peak at δ 6.8 ppm proves that chalcone is successfully incorporated into the system. The IR and NMR spectrum of other two compounds **a11** and **a111** are almost same as compound **a1** since compounds **a11** and **a111** were synthesized by replacing the second and third chlorine atom by chalcone from the cyanuric chloride, respectively.

UV analysis: Fig. 3 shows the UV-visible spectrum of compound **a1**, which represent the series containing 2,4-dichloro-1-one(4-hydroxyphenyl)phenone based chalcones. The UV visible spectrum of the synthesized compound reveals two peaks centered around 245 nm and 320 nm, which can be due to the absorption of aromatic and π–π* transition of >CH=CH<, respectively. The UV absorption values of all the synthesized compounds are fallen in these two regions.

Antimicrobial activity: The MIC values are reported in Table-1 showed that all the synthesized compounds were active against *Escherichia coli* than *Staphylococcus aureus*. There

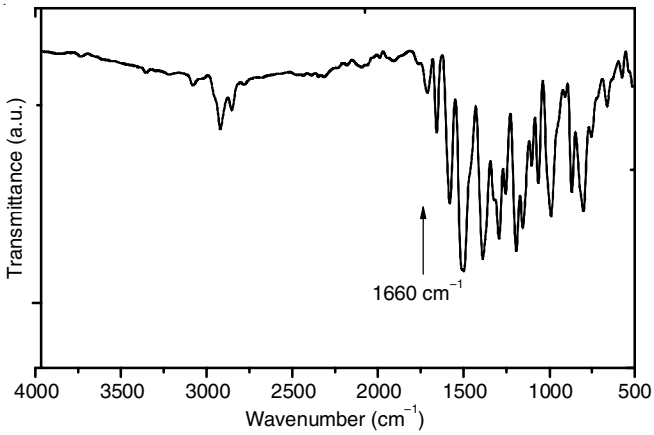


Fig. 1. FT-IR spectrum of **a1**

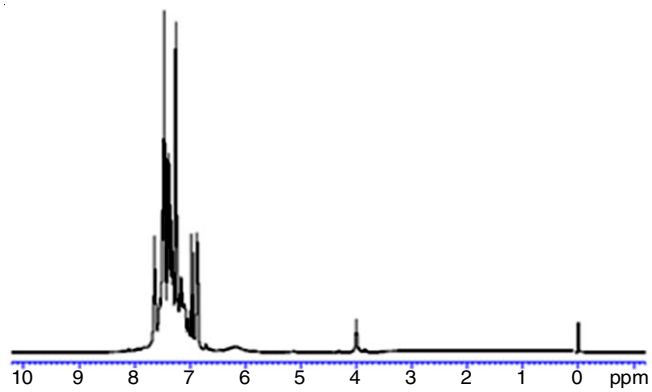


Fig. 2. ^{1}H NMR spectrum of **a1**

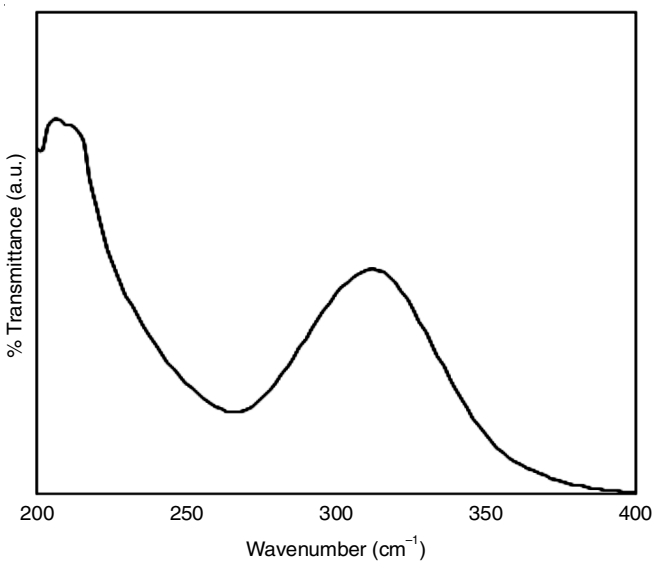


Fig. 3. UV spectrum of **a1**

TABLE-1
MIC VALUE (µg/mL) AND THE MOLECULAR WEIGHT OF THE SYNTHESIZED COMPOUNDS

Compound code	m.w. (g/mol)	MIC (µg/mL)	
		Staphylococcus aureus	*Escherichia coli*
Cyanuric chloride	184.46	31.25 ± 2.55	31.25 ± 1.92
Ciprofloxacin	331.3	7.81 ± 1.26	3.91 ± 0.39
a1	441.1	15.63 ± 1.27	7.81 ± 0.56
a11	697.0	15.63 ± 1.68	3.91 ± 0.37
a111	954.1	31.25 ± 3.01	1.95 ± 0.11

was a remarkable difference in MIC values between the parental cyanuric chloride and synthesized compounds. This suggests that the presence of chlorine atoms in chalcone to certain extent enhances the antimicrobial activity in the case of Gram-negative bacteria. However, it is not the only reason for the enhancement in the activity of the drug, since the drug activity on Gram-positive bacteria showed a reverse trend. The difference in the activity of the drug towards two different bacterial stain is due to the constituents of the cell wall or the binding ability of the synthesized compounds to that of DNA gyrase protein/topoisomerase IV of microorganisms. The synthesized compounds showed higher activity than the parent cyanuric chloride indicate the high availability of chlorine in those compounds.

Structure activity relationship (SAR): The antimicrobial activity of synthesized compounds **a1**, **a11** and **a111** on Gram-negative bacteria (*E. coli*) are increased in the order of **a111** > **a11** > **a1** > cyanuric chloride. This shows that the ability of compound to destroy the Gram-negative bacteria are increased with increases in the bulkiness of molecule. It is noted that chalcone, 2,4-dichloro-1-one(4-hydroxyphenyl)phenone contains two chlorine atoms in its molecular structure. Replacing one chlorine from the cyanuric chloride by chalcone molecule increases the net chlorine content by one unit and henceforth, the activity increased with increase in the substitution. The observed MIC value suggested that an increase of chalcone moiety in the molecular structure plays an important role on antimicrobial activity. The chlcone molecule preferentially bonded with the gyrase protein thereby destroying the microorganism. However, on Gram-positive bacteria, an activity showed a reverse trend, which can be expected due to the low sensitivity of gyrase protein towards the synthesized compounds as reported earlier for similar systems [21]. A high chlorine content molecule, compound **a111** has a similar activity (Fig. 4) with that of parent cyanuric chloride (31.25 µg/mL). Therefore, an activity of the synthesized compounds behaved differently with different bacterial stains. This data also proves that the activity of these molecules on microbes is not only by endocytosis process which is depending on the size of the active drug molecules. Therefore, enhanced activity by increasing the substitution is due to the protein binding ability of the substituted molecules.

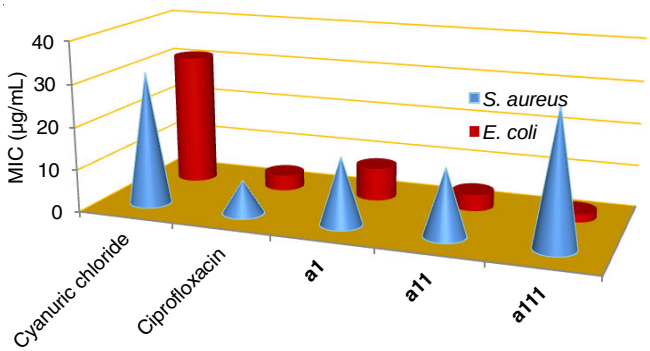


Fig. 4. Comparative graph of **a1**, **a11** **a111**, and cyanuric chloride on Gram-negative bacteria

Docking studies: A high observed antimicrobial activity of the synthesized compounds (**a1**, **a11**, **a111**) drives us further to explore the possible interaction (docking) between the molecule and the active sites of DNA gyrase protein (PDB: 1KZN). The DNA gyrase protein plays a major role in the cell for their replication, transcription and it can catalyze the negative super coiling of the bacterial circular DNA. Therefore, binding the drug with the DNA gyrase protein can lead to the cell death. Enzymatic bustle inhibition and covalent enzyme-DNA gyrase poisoning stabilization is the supreme reason for the greater antimicrobial activity of the drugs targeting gyrase [22].

In the present study, *in silico* approach on synthesized compounds (**a1**, **a11**, **a111**) against DNA gyrase is carried out using virtual screening, molecular docking, and ADMET methods. Virtual screening of synthesized compounds **a1**, **a11** and **a111** showed the binding affinity towards target BRCA2 (which is coming under topoisomerase II type/ gyrase protein). The compounds were screened with binding affinity and the compounds were selected as hits. The binding pocket of the enzyme is represented in Table-2.

TABLE-2
DNAgyrase BINDING SITE

Ligands	Amino acids binding site in the protein
a1	Asp73, Asn46, Ile78, Vall67, Vall20, Val71, Thr165, Glu50, Val43, Ile90, Ala96, His95 & Ser121
a11	Asp73, Gla50, Thr165, Asn46, Val71, Ile73 Val43, Val167, Ile90, Ala96, Val113, Tyr36, Gly117 & Met35
a111	Val93, Leu16, Thr92, Asp17, Pro150, Val89, Val49, Val84, Leu154, Pro153, Gly83, Gln88 & Ser85

Figs. 5 to 8 represents the docking of DNA gyrase with compounds **a1**, **a11** and **a111**. In these figures, the dark green line indicated hydrogen bonding between the atoms and the other interaction are hydrophobic. The docking values for compounds **a1**, **a11** and **a111** was found to be -8.3020, -8.9366, and -9.1900 Kcal/mol, respectively. The molecular docking value showed the binding mode and interaction energy. The order of binding energy was **a111** > **a11** > **a1**. Among the different compounds, compound **a111** was the best affinity than other two compounds. The affinity of molecule having topoisomerase II protein increases with increase in chlorine content/chalcone moiety, which in turns increases the antimicrobial activity of the compounds. Compound **a111** has got highest binding energy and therefore showed the excellent MIC value of 1.95 µg/mL for *Escherichia coli*. However, for Gram-positive bacteria, the pattern of correlation between binding energy with that of MIC is not clear which can probably due to the resistivity provided by *Staphylococcus aureus* and or the drug molecule may try to bind with topoisomerase II and IV simultaneously [21]. Using this docking study, it is clear that synthesized compound is preferentially bonded with the gyrase protein (topoisomerase II) and thereby destroying the Gram-negative bacteria.

Conclusion

Novel cyanuric chloride derivatives **a1**, **a11** and **a111** were synthesized and characterized. The antibacterial activity of compounds **a1**, **a11** and **a111** was tested on *Staphylococcus aureus* and *Escherichia coli*. The synthesized compound **a111** showed excellent activity towards the tested bacterial stains *Escherichia coli* having a value of 1.95 µg/mL. The order of binding energy of the synthesized compounds was **a111** > **a11**

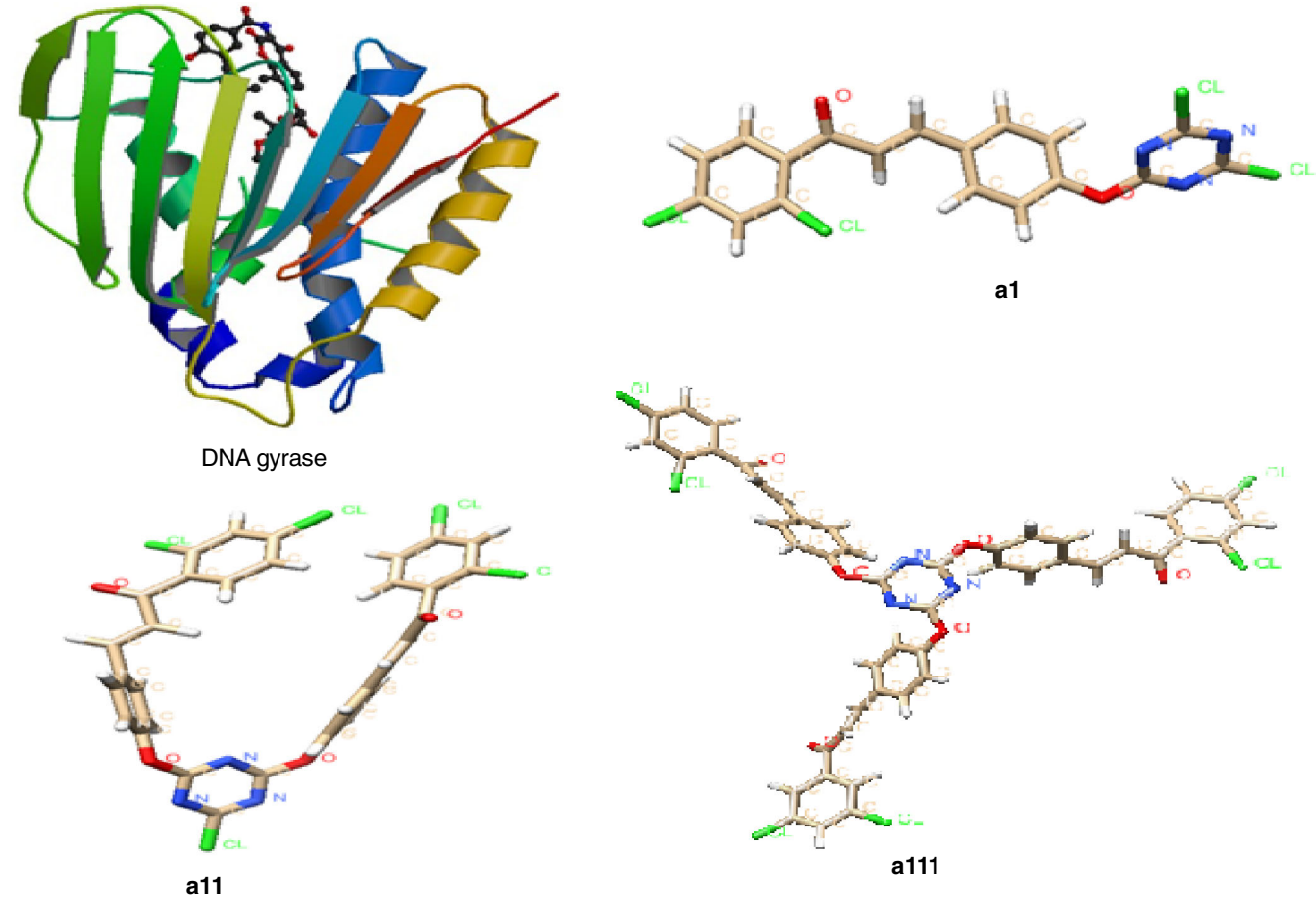


Fig. 5. 3D Structure of target protein DNA gyrase and test ligands (**a1**, **a11** and **a111**)

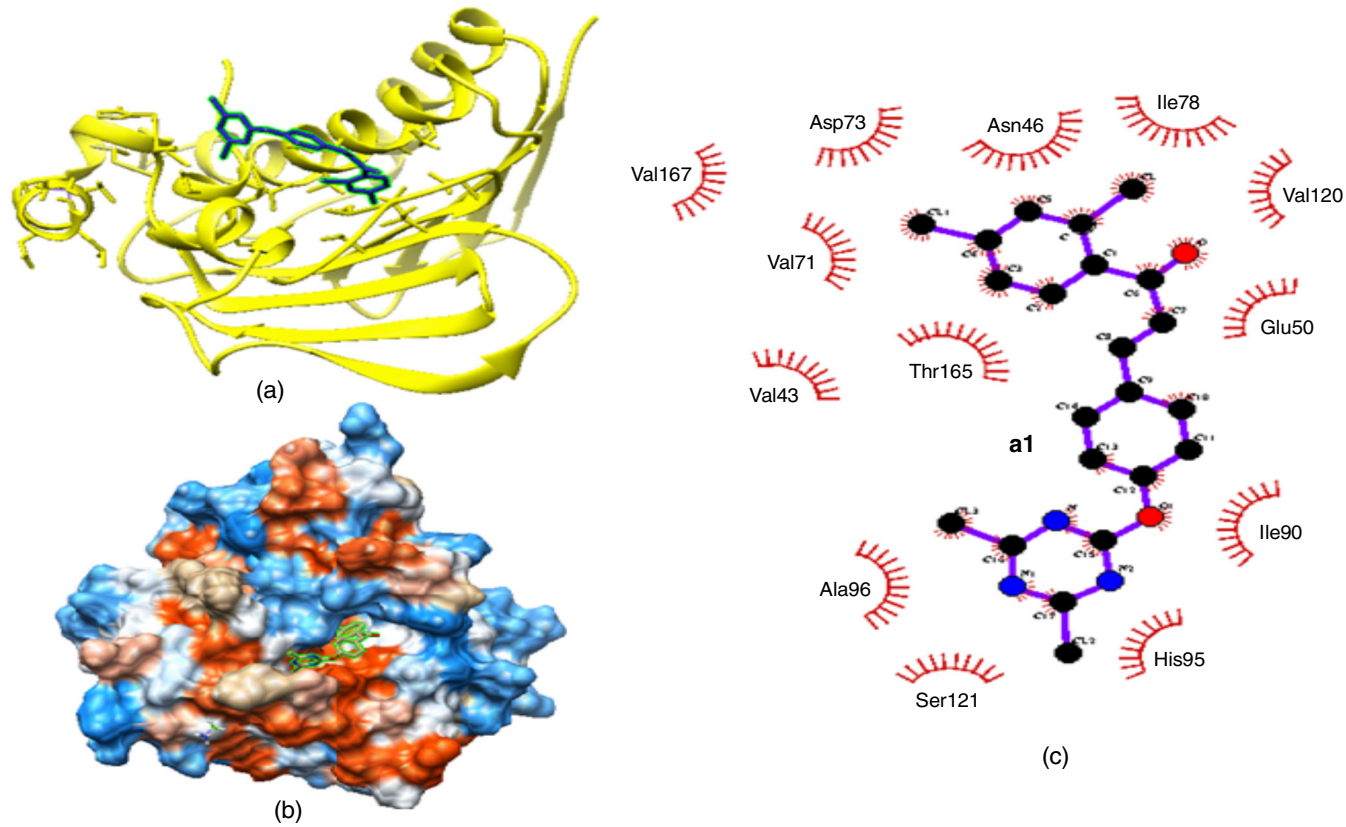


Fig. 6. (a) Docking of **a1** (b) electrostatic surface of DNA gyrase alongside the amino acids in the active site of DNA gyrasemotif with **a1** (c) interaction of **a1** with an amino acid residue of the homologous regions and the active sites of DNA gyrase

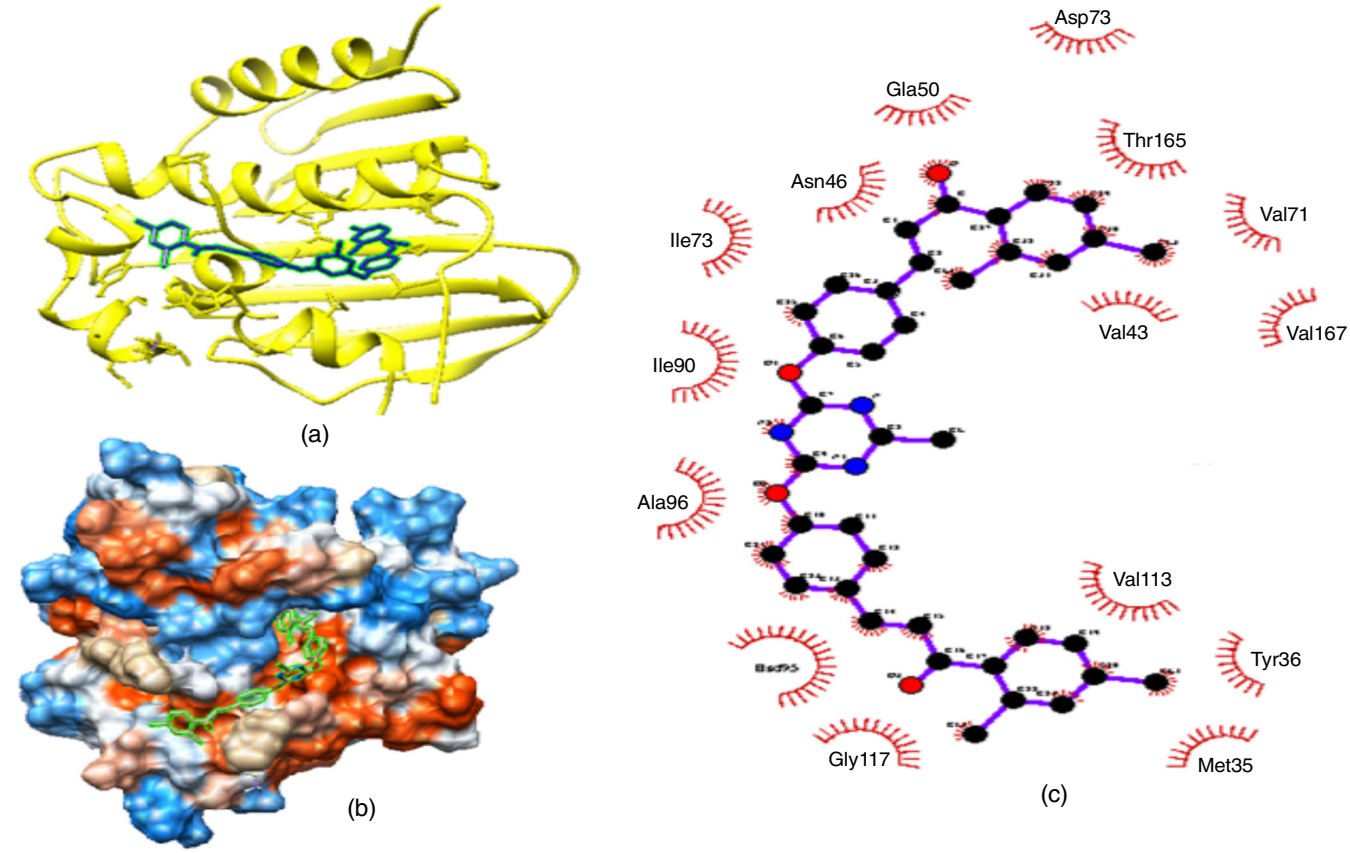


Fig. 7. (a) Docking of **a11** (b) electrostatic surface of DNA gyrase alongside the amino acids in the active site of DNA gyrasemotif with **a11** (c) interaction of **a11** with an amino acid residue of the homologous regions and the active sites of DNA gyrase

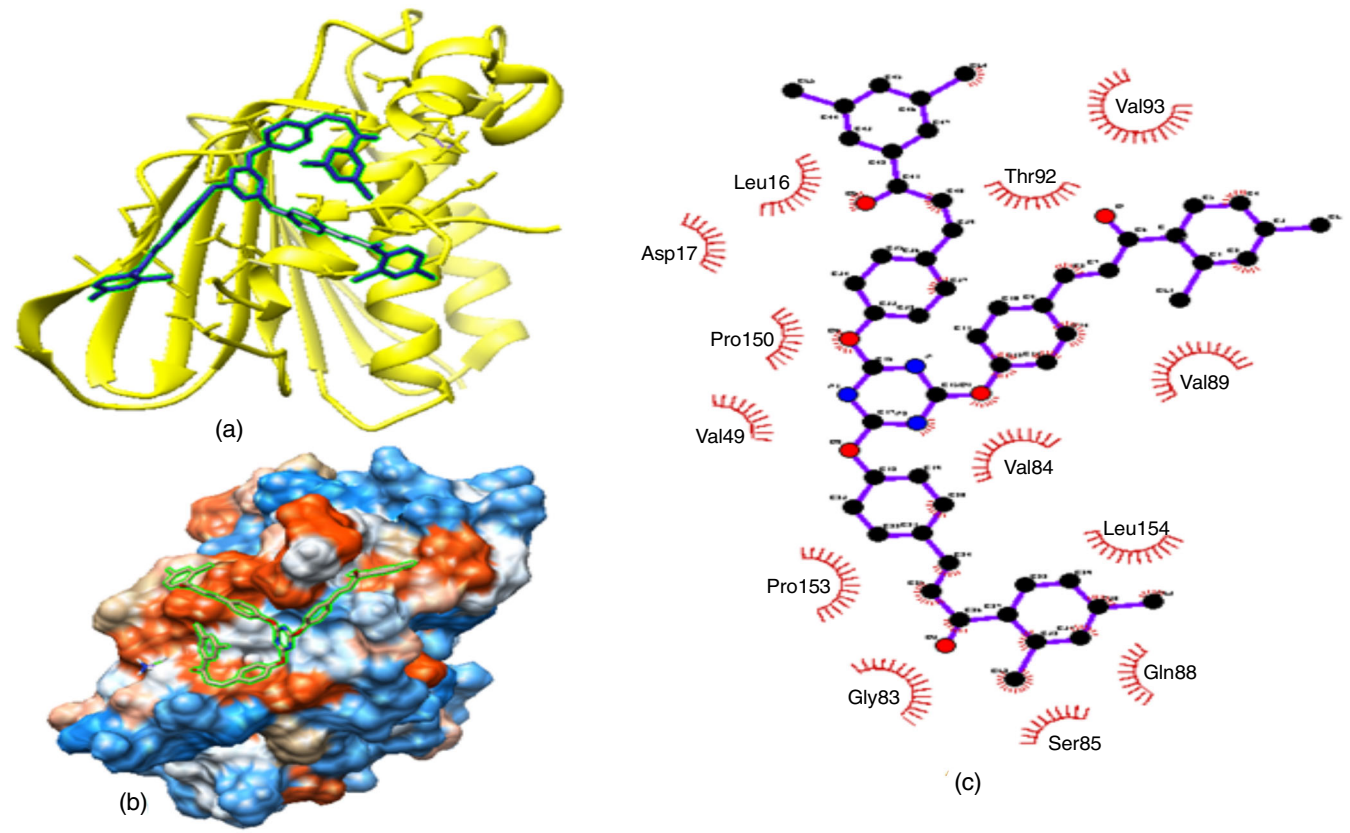


Fig. 8. (a) Docking of **a111** (b) electrostatic surface of DNA gyrase alongside the amino acids in the active site of DNA gyrase moiety with **a111** (c) interaction of **a111** with an amino acid residue of the homologous regions and the active sites of DNA gyrase

> **a1**. Compound **a111** shows the best affinity than other compounds this was found out by *in silico* molecular docking method.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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