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ARTICLE TYPE

Studies on the synthesis of sugar triazole based ligand for protein and DNA binding

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Bis-triazole derivatives accomplished by [3+2] cycloaddition methodology were well characterized. Interaction of bis-triazoles with BSA and CT-DNA had good correlation with experimental and docking studies. Compounds showed moderate to excellent antibacterial activity.

1. Introduction

Saccharides and proteins, such as liposaccharide and phenolic glucosidases respectively have a wide range of applications in the field of materials, biology and drug design.[1-5] Among the sugar derivatives, *C*-disaccharides, *C*-aryl glycosides, *C*-glycosidically linked ADP glycerol *β*-**D**-manno-heptose analogues[6-11], C-nucleosides etc., have been reported as non-hydrolyzable mimics of *N*- and *O*- glycosides which were used in designing new chemotherapeutic entities.[12,13] In particular, alpha, beta-unsaturated *β*-*C*-glycosidic ketones of **D**-glucose have served as the core moiety in natural ligands for making covalent bonds with many receptors, such as the peroxisome proliferator-activated receptor g (PPARg).[14]

Chalcone and their derivatives were found to be well-known intermediates for the synthesis of various heterocyclic compounds[15] which possess antimicrobial, analgesic, antimalarial, anticancer[16-19] activities etc., Some of the aromatic based (*E*)-chalcones (1,3-diphenyl propen-1-ene) have led to the search for novel eco-friendly alternatives to conventional nematicidal compounds.[20] In particular, isoliquiritigenin, a chalcone derivative (**Figure 1**) which was found in lico-rice and shallots[21-23] have cytotoxic activities towards various cancer cell lines.[24] It inhibits epidermal ornithine decarboxylase induction and ear edema formation due to its anti-tumour promoting action. Moreover, it also has some biological activities like anti-platelet aggregation, anti-oxidant inhibition of aldose reductase and cardiac effects.[25-27]

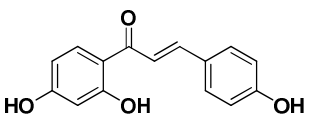


Figure 1 Structure of *E*-chalcone (Isoliquiritigenin).

Owing to the broad range of applications in biochemical, pharmaceutical, material sciences and biological applications like antibacterial, anti-inflammatory, antihypertensive, antifungal, anticancer[28-31] activities, the 1*H*-1,2,3 triazoles have attained much attention in synthetic organic chemistry.[32-35] Similar biological functions were also reported for a variety of *bis*-triazole compounds.[36] In the past few years, the use of Cu(I) catalyzed azide-alkyne cycloaddition reaction has provided a new insight in the field of carbohydrate research.[37, 38] In addition, *bis*-triazole derivatives play a major role in drug discovery in targeting mRNAs coding for proteins[39] and were also used as potent HIV-1 protease inhibitors.[40]

The diverse biological activities possessed by chalcone and triazole derivatives as reported in the literature inspired us to look for the interaction of chalcone based triazole (ligand) with serum albumin, a major serum protein responsible for the transportation of various compounds including drug to the target site.[41] Generally, protein ligand is a protein binder, a molecule which can bind to a specific site on a protein. Small ligand-protein underlies many fundamental processes in biology and in turn forms the basis for pharmacological intervention of human diseases in medicine. Though there are some *in vivo* methods for the detection of ligand-protein interaction[42], herein we report the use of spectroscopic techniques to understand the binding of ligand to the protein which was further supported by docking studies.

An interesting group of chemotherapeutic agents used in cancer therapy includes the molecules that interact with DNA or site specific cleavage of DNA strand, alkylation of DNA; thereby interfering with the replication and transcription machinery.[43, 44] Ligands in the metal complexes play a major role in binding to DNA. It is well known in the literature that planar ligands promote intercalative binding of the complex to DNA[45-48] whereas non-planar ligands promote groove binding [49]. In continuation of our ongoing research in the area of saccharide chemistry[50-53], the present investigation focuses on the effect of Cu(I) catalyzed pathway chosen to synthesize sugar-chalcone based 1,2,3-triazoles which serves as a ligand for binding both protein and DNA.

2. Results and Discussion:

2.1 Synthesis and characterization:

4,6-*O*-Butylidene-**D**-glucopyranose was synthesized from **D**-glucose by adopting the literature procedure.[54, 55] *β*-*C*-Glycosidic ketones were synthesized by the Knoevenagel condensation of 2,4-pentanedione with sugar derivatives, such as 4,6-*O*-butylidene-**D**-glucopyranose, **D**-xylose and **D**-glucose in the presence of sodium bicarbonate using THF-H_2O as solvent.[56-58] In addition, the sugar-azide was also synthesized by following the literature procedure.[59] *O*-alkylation of 3,4-dihydroxybenzaldehyde and 2,4-dihydroxybenzaldehyde with propargyl bromide using K_2CO_3 as base resulted in *bis*-*O*-propargylated aldehydes, **2a** and **2b**.

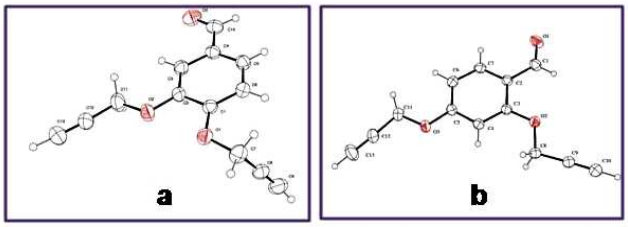
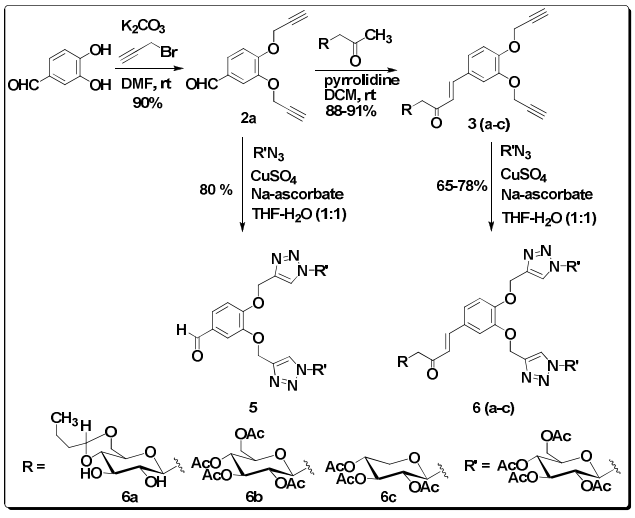
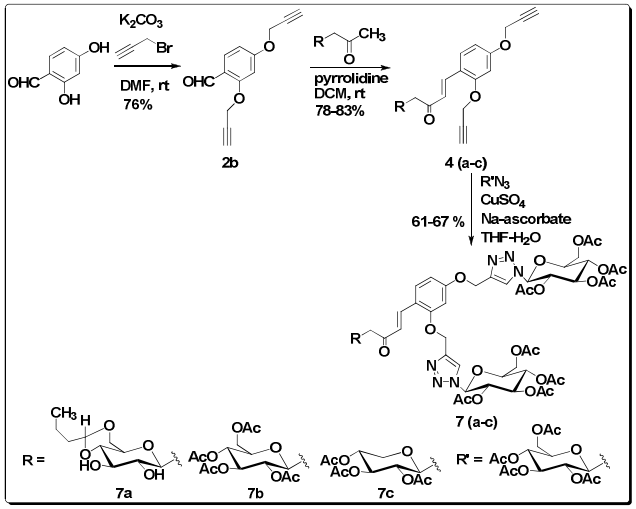


Figure 2 ORTEP view of *bis*-*O*-propargylated derivatives, (a), **2a**; (b), **2b**.

Bis-propargylated derivatives, **2a** and **2b** were crystallized in chloroform. ORTEP view of compounds **2a** and **2b** are shown in **Figure 2**. Aldol condensation of various *β*-*C*-glycosides **1**(**a-c**) with two different positional isomers of dihydroxypropargylated benzaldehyde **2a** and **2b** using pyrrolidine as catalyst resulted in 79-91% of the corresponding alpha, beta-unsaturated compound **3**(**a-c**)/**4**(**a-c**). "Click reaction" of sugar azide with dihydroxypropargylated sugar-chalcone using tetrahydrofuran and water as solvent in the ratio of 1:1 resulted in excellent yield of *bis*-triazole derivatives. The simple aromatic *bis*-propargylated-aldehyde crystallized whereas after the incorporation of the sugar moiety, the compound failed to crystallize. The crystallographic data and the hydrogen bonding interaction of **2a** and **2b** are shown in **Table 1**,**2** and **Figure 3**,**4** in **ESI**.



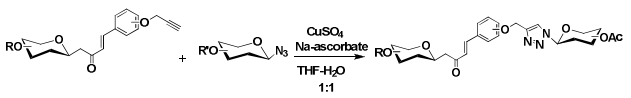
Scheme 1 Synthesis of bis-triazole derivatives, **6** (**a-c**)



Scheme 2 Synthesis of bis-triazole derivatives, **7** (**a-c**)

The formation of *bis*-propargylated aldehyde was confirmed from both [^{1}H and ^{13}C] NMR spectroscopy. The appearance of multiplet in the range δ 2.41-2.51 ppm in ^{1}H NMR spectrum of compound, **2a** corresponds to the two acetylenic protons. The product formation was further confirmed from the appearance of four signals at δ 77.7, 77.5, 76.7, 76.5 ppm in the ^{13}C NMR spectrum which belongs to the acetylenic carbons. From the ^{1}H NMR spectrum of aldol condensed product, **3a**, the *trans*-alkene proton was observed as two doublets at 7.6 and 6.7 ppm with *J* value of 15.9 and 16.2 Hz each respectively. Furthermore, the appearance of signals at 144 and 125 ppm corresponds to the *trans*-alkene carbon confirms the formation of the product. The structures of the resulting sugar-chalcone based *bis*-triazole derivatives **6**(**a-c**) and **7**(**a-c**) were determined by ^{1}H and ^{13}C NMR spectroscopy, mass spectroscopy and elemental analysis. From ^{1}H NMR spectrum of compound **6a**, the Ar-*H* proton appears in the region 7.26-7.01 ppm whereas the *trans* alkene peak was observed as two doublets at 7.52 and 6.67 ppm with a large coupling constant value of J ~ 16 Hz. Since the molecule is unsymmetrical, the two triazole protons appeared as two singlets at 8.33, 8.24 ppm and its corresponding carbons were observed at 122.0 ppm respectively. This observation confirmed the formation of *bis*-triazole product. The ^{1}H NMR spectrum of **6a** notably exhibited a large coupling constant for H-1 signal ($J_{H1,H2}$ = 9.0 Hz), indicating a *trans*-diaxial orientation of H-1 and H-2 as expected for a *β*-D-configured glucopyranose moiety.[60,61] The difference between the chemical shift of C4 and C5 carbon was found to be 22.54 and 22.68 which clearly confirmed the formation of 1,4-disubstituted-*bis* triazole derivative. Reaction time, product yield and spectroscopic data are given in **Table 1**.

Table 1 Synthesis of sugar-chalcone based unsymmetrical *bis*-triazole derivatives



Compound No.	Time (h)	Yield (%)	δAlk-*H* (ppm), $J_{H1,H2}$ Hz	δTrz-*H* (ppm)
5	24	80	-	8.33, 8.18
6a	16	78	7.50, 15.9	8.33, 8.24
6b	24	65	7.47, 15.9	8.35, 8.23
6c	18	73	7.40, 16.2	8.27, 8.15
7a	26	67	7.87, 16.1	8.12, 8.04
7b	20	61	7.84, 16.2	8.02, 7.99
7c	24	64	7.84, 16.3	8.09, 8.07

2.2. Fluorescence Measurement:

2.2.1. BSA binding studies:

Fluorescence measurements are very important in getting information about the binding mode, binding constants and binding sites of the small molecule attached to the protein. Fluorescence titration experiments were carried out at room temperature with Bovine serum Albumin (BSA) in the presence of triazole derivatives, **5**, **6a** and **6c** to determine the binding constant that reflect the extent of binding between the triazole derivatives with BSA. A solution of BSA (1 μM) was titrated with various concentrations (0-6 μM) of the test compounds, **5**, **6a** and **6c**. Fluorescence spectra were recorded in the range 320-440 nm where the maximum fluorescence emission of BSA was observed at 370 nm upon excitation at 270 nm (**Figure 5**). The effect of all the triazole derivatives on the fluorescence emission spectra of BSA are shown in **Figure 5**. In all the three cases, the fluorescence intensity of BSA decreased significantly by the gradual addition of *bis*-triazole derivatives, **5**, **6a** and **6c** are represented in **Figure 5**. Thus, the hypochromic shift was observed in all the cases whereas it is more predominant in the case of **6c**. These results show a strong interaction of BSA with all the three sugar-chalcone based *bis*-triazole derivatives. Moreover, among the three derivatives, **5**, **6a** and **6c** titrated with BSA, compound, **6c** was found to have excellent binding with the protein, thus resulting in higher quenching. This may be due to the difference in the sugar moiety. Thus it is obvious from the result that compared to the glucose derivative, the xylose coupled with triazole, **6c** found to have better binding with the protein, BSA.

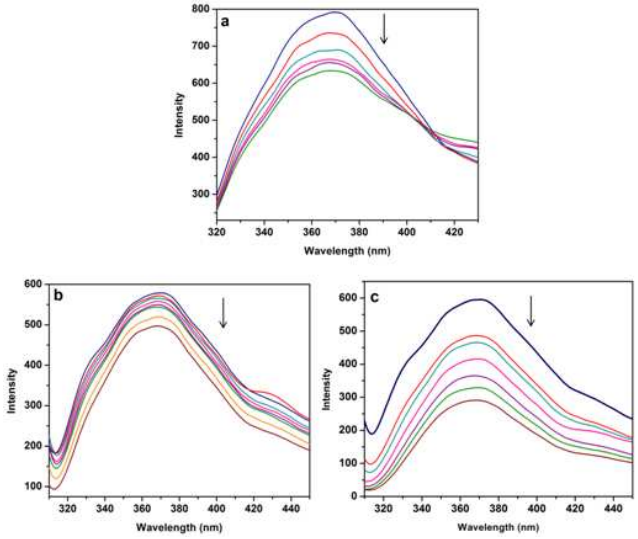


Figure 5 Fluorescence titration spectra of compounds (0-6 x 10^{-6} M) with BSA protein (1 x10^{-6} M) at 25 °C, (a), **5**; (b), **6a** and (c), **6c**.

In order to determine the quenching property, the fluorescence decay data were analyzed via the Stern-Volmer equation $F_0/F=1+ K_{sv}[Q]$ where, K_{sv} the Stern-Volmer quenching constant, F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher respectively. [Q] is the concentration of quencher and K_{sv} is the Stern-Volmer quenching constant which in turn was obtained by plotting F_0/F vs [Q] [**See ESI for details**]. According to the equation, $K_{sv} = Kq.\tau_0$, where *K*q is the quenching rate constant and τ_0 is the fluorescence lifetime of protein in the absence of quencher, the value of τ_0 is considered to be 10^{-8} s. For three derivatives **5**, **6a** and **6c** the values of quenching rate constant (Kq), due to the binding of BSA with the sugar-based *bis*-triazole derivatives was found to be $1.54\pm0.1x10^7$ mol·L^{-1}·s^{-1}, $0.19\pm0.3x10^7$ mol·L^{-1}·s^{-1}, $2.1\pm0.4x10^7$ mol·L^{-1}·s^{-1} respectively. These results revealed the formation of a complex between BSA and sugar-based *bis*-triazole derivatives. When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation, $\log(F_0–F)/F=\log K+n \log[Q]$, where K is the binding constants to a site and n is the number of binding sites per BSA. The binding constant (K) for all the three sugar derivatives **5**, **6a** and **6c** were obtained as $6.08\pm0.1x10^4$ L·mol^{-1}, $3.1\pm0.3x10^4$ L·$mol^{-1}$and $1.22\pm0.4x10^5$ L·mol^{-1} respectively which indicate that the binding force for compound **6c** was comparatively higher with BSA than for the other two triazole derivatives. Similarly, the number of binding sites (n) for derivatives, **5**, **6a** and **6c** were calculated as 1.53, 0.99 and 1.18 respectively. The values of n are approximately equal to 1 for derivatives **6a** and **6c**, indicating that there is only one class of binding site, whereas derivative **5** has two binding sites which may be due to the hydrogen bonding nature of aldehyde in compound **5** with amino acid residues in BSA. These experimental results were in good correlation with the docking analysis.

2.3 UV measurements:

2.3.1 DNA binding studies:

Electronic absorption spectroscopy is a usual way to

investigate the interaction of ligand (triazole derivatives) with DNA. The binding studies were carried out for three different sugar triazole derivatives (**5**, **6a** and **6c**) in Tris-HCl buffer (50 mM, 7.5 pH) using DMF-H_2O as solvent mixture. A solution of calf thymus DNA (CT-DNA) in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8-1.9 indicating that the DNA was sufficiently free of protein. The derivatives were titrated with calf thymus DNA and the absorption bands are shown in **Figure 6**. The concentration of CT-DNA was determined from the absorption intensity of 260 nm with ε value of 6600 $M^{-1}cm^{-1}$. Absorption titration experiment was performed with different concentrations of DNA from 10-150μm by keeping the concentration of ligand constant (10 μm). The absorbance bands were followed for each addition of DNA. In order to eliminate the absorbance that arise due to CT-DNA, an equal amount of the same was added to both the compound as well as the reference solution. The experiment was conducted thrice maintaining all the parameters constant and the error bar is represnted in the linear plots.

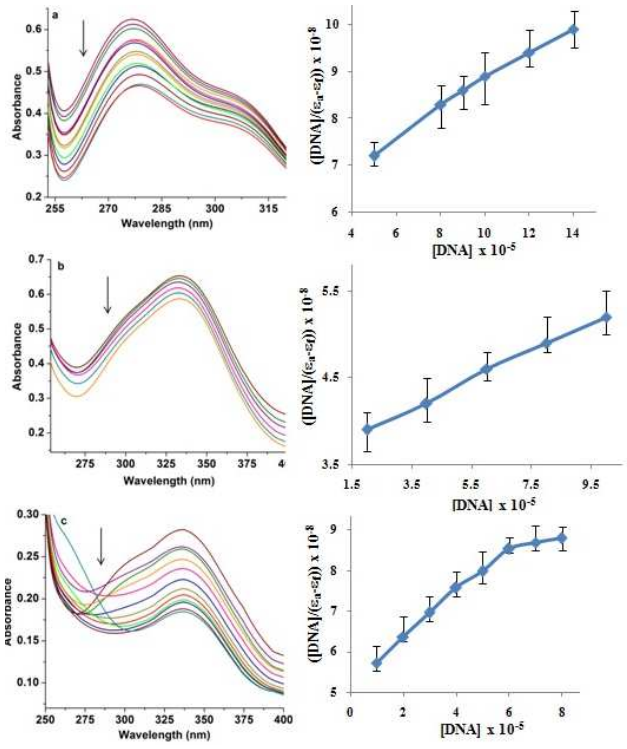


Figure 6 Absorption titration spectra of compounds (10 x 10^{-6} M) with CT-DNA (0-150 x10^{-6} M), (a), **5**; (b), **6a** and (c), **6c**; The arrow shows the change in the absorbance upon increasing amount of CT-DNA; Right side plot of ([DNA]/ (ε_a-ε_f)) versus [DNA].

The electronic absorption spectra of ligands, **5**, **6a** and **6c** consist of well resolved band in the region 250-400 nm. The band that were found around 275 nm in the case of **5** and the band that was observed around 325 nm for the ligands, **6a** and **6c** may be attributed to π-π* transition respectively. Upon the addition of CT-DNA, the above bands corresponding to ligands, **5**, **6a** and **6c** showed significant hypochromism with slight red shift. The observed changes in the absorbance of bands with increasing concentration of CT-DNA (**Figure 6)** reveal the structural alteration of the ligand upon binding to DNA. The hypochromic effect is also suggestive for the compounds possessing higher propensity for DNA binding. It indicates that the interaction of the compounds, **5**, **6a** and **6c** takes place by the direct formation of a novel complex with double helical DNA. It is well known that only a planar compound has the ability to intercalate with DNA. Since the sugar-based triazole derivatives were non-planar, they do not prefer to intercalate with DNA and are found to be non-intercalators. It is interesting that the methyl group of the protecting group in the saccharide moiety increases the DNA binding affinity by exhibiting favorable hydrophobic interaction on the surface of the DNA major groove.

In order to compare quantitatively the binding strength of the three triazole derivatives with CT-DNA, the intrinsic binding constant (K_b) was calculated from the following equation.[62]

$$[DNA]/|\varepsilon_a-\varepsilon_f| = [DNA]/|\varepsilon_b-\varepsilon_f| + 1/K_b|\varepsilon_b-\varepsilon_f|$$

where, [DNA] represents the concentration of DNA, ε_a, ε_f, ε_b correspond to A_{obsd}/[compound], the extinction coefficient of the free ligand and the extinction coefficient of the ligand in the fully bound form with DNA respectively. [DNA]/[ε_b-ε_f] vs [DNA] gave a slope and the intercept which are equal to 1/[ε_a-ε_f] and (1/K_b)[ε_b-ε_f] respectively. In the plot of [DNA]/ ε_a-ε_f versus [DNA], K_b is obtained from the ratio of the slope to the intercept.

The magnitudes of the intrinsic binding constants (K_b) for compound **5**, **6a** and **6c** were calculated to be $5.7\pm0.2x10^3$ M^{-1}, $6.1\pm0.2x10^3$ M^{-1} and $9.2\pm0.1x10^3$ M^{-1} respectively. The observed values of the binding constant (K_b) revealed that the ligand, **6c** bound strongly with CT-DNA compared to the other two ligands and the order of binding affinity is **5** < **6a** < **6c**. The observed hypochromism of all the three ligands and their binding constant values suggests that the reported ligands have a weaker binding with DNA than the classical intercalators and it is likely to bind with CT-DNA *via* groove mode. Since the synthesized derivatives are non-planar in nature theoretically it is expected that the ligand prefer to have groove binding with DNA rather than intercalation. Moreover, docking analysis supported the experimental results and further revealed the nature of binding as major groove binding. Though the triazole derivative showed a strong hypochromism with DNA, the observed moderate binding constant and the non-planar nature of the molecule gave a strong view that the mode of binding of the triazole derivatives with CT-DNA as major groove binding. In addition, the result was further supported by the docking results.

While interacting the ligands with BSA and CT-DNA, there cannot be any chemical changes. It is because the ligand interacts with amino acid through weak hydrogen bonding in the case of BSA, whereas in CT-DNA, the ligand acts as a guest molecule in the major groove. In addition, there cannot be any conformational changes in both BSA and DNA while interacting with the synthesized compound. The change in the electrochemical properties of the compound with BSA and CT-DNA has been studied using the cyclic voltammetry and the details about the studies are provided in **ESI**.

2.4 Docking analysis:

There has been increasing interest in the usage of docking

methods to study binding of molecules to proteins and DNA. Geometry optimization has been carried out for three different sugar triazole derivatives (**5**, **6a** and **6c**) using PM6. The optimized structures have been used for molecular docking analysis. The docking of sugar triazole derivatives with BSA (PDB ID: 4F5S) and duplex DNA d $(CGCGAATTCGCG)_2$ dodecamer has been performed to understand the nature of interaction between sugar triazole derivatives with BSA and DNA. The grid box with dimensions of 82 x 76 x 70 Å and 80 x 80 x 112 Å has been used for BSA and DNA, respectively. In the docking analysis, receptor was treated as a rigid body. In Auto dock vina, non-polar hydrogen atoms were removed from the receptor and their partial charges were added to the corresponding carbon atoms. The most favorable docked orientation was depicted in **Figure 7** and **9**.

2.4.1 In-silico docking

All the compounds used for docking showed best fit Root Mean Square deviation value of 0.00 with BSA and DNA. Binding affinity ranges of the compounds with BSA and DNA were given in **Table 2**. It was very obvious from the result that all the compounds bound to BSA with a greater affinity than DNA. Furthermore, the mode of binding of all the three sugar-triazole ligands (**5, 6a** and **6c**) with BSA was revealed from **Figure 7** and studies of the synthesized ligands clearly showed the importance of mode of action through the interaction between sugar and targeted protein (BSA) and DNA. The interaction of all the three sugar-triazole ligands with amino acid residues of BSA were given in **Figure 8**. It can be seen from the **Figure 8** that the residues Leu112, Asp113, Lys144, Leu115, Arg185, Arg458 His145 Ser 428, Leu189, Arg427, Thr421, Pro420, Glu424, Lys431, Leu462, from the BSA were interacting with the ligands. From **Figure 9**, it can be seen that the ligands bound to the major grooves of DNA. In addition, all the three ligands were docked with DNA by altering the distance of major groove nucleobases to check the extent of binding of the ligands through intercalative mode. The molecular modeling study shows that all the three ligands bind DNA preferably through groove mode.

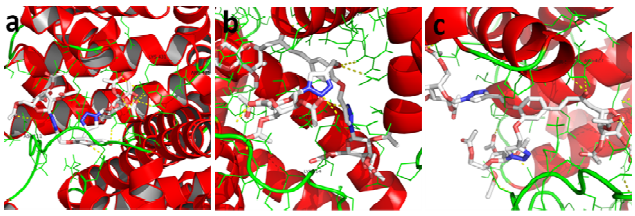


Figure 7 The docked pose of the compound (a), **5**; (b), **6a** and (c), **6c** in the active site of BSA.

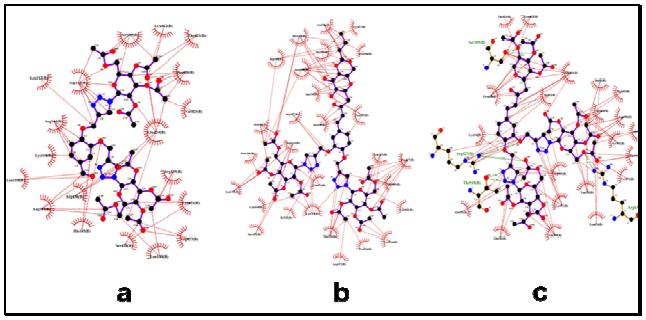


Figure 8 Model for amino acid interaction of compounds with BSA, (a), **5**; (b), **6a** and (c), **6c**.

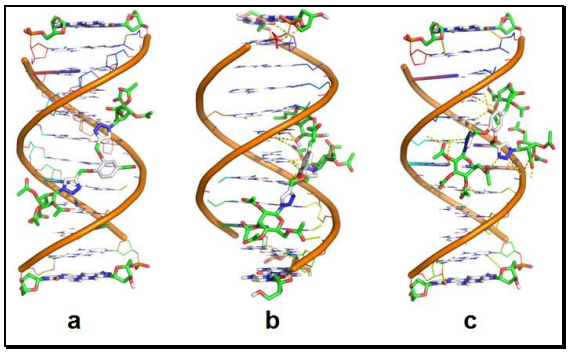


Figure 9 The docked pose of compound with CT-DNA, (a), **5**; (b), **6a** and (c), **6c**.

The binding affinity of all the three *bis*-triazole derivatives were compared between experimental and docking studies and the comparable results were represented in **Table 2**.

Table 2 Binding affinity of *bis*-triazoles from docking and experimental studies.

Compound No	Binding affinity result from docking studies		Binding affinity result from experimental studies	
	BSA	DNA	BSA	DNA
5	-9.2 to -8.3	-7.2 to -6.7	$6.08X10^4$	$5.7X10^3$
6a	-8.0 to -9.0	-7.7 to -7.4	$3.1X10^4$	$6.1X10^3$
6c	-9.5 to -8.9	-8.2 to -7.7	$1.22X10^5$	$9.2X10^3$

2.5 Antibacterial studies:

A screening of antibacterial activity of compounds **5**, **6a** and **6c** were tested against four human pathogenic bacteria namely *Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* by the agar diffusion method. All the three compounds were tested, each at 25µL, 50µL and 75µL (1%) concentration to test their efficacy in inhibiting the growth of the tested pathogens (human pathogen). The antibacterial activity of these compounds were dose dependent and were found to be significant at 75 µg/mL concentration [**See ESI for more details**]. The experiment was conducted in triplicate and the error bar is represented in the bar graph (**Figure 10**).

The antibacterial activity of the *bis*-triazole derivatives are

represented by the plot of percentage of inhibition by various bacterial strains (**Figure 10)**. From the graph it is found that among the three triazole derivatives, **5**, **6a** and **6c** studied, compound **5**, which posses only triazole derivatives showed moderate to good activity against all the bacteria tested, whereas the compound, **6a** and **6c** which possess the sugar-chalcone coupled with triazole were found to exhibit superior activity against *Streptococcus pyogenes* and *Bacillus subtilis*. Thus, from the result it is found that the sugar-chalcone based *bis*-triazole derivatives exhibit excellent antibacterial activity. It is also found that the xylose moiety in the product plays a significant role because the compound, **6c** found to inhibit all the bacteria tested and the inhibition found to be better compared to the other two derivatives. Compound **6a** which has a partial protected sugar-triazole derivative showed minimum inhibition with *Klebsiella pneumonia* whereas it exhibited moderate activity against *Pseudomonas aeruginosa*. Thus it is observed from the result that the antibacterial activity of sugar-based triazole derivatives depends on both the sugar-chalcone as well as the triazole moieties. Among the three sugar triazole derivatives tested for antibacterial activity, excellent inhibition was observed for all the compounds, **5**, **6a** and **6c** against *bacillus subtilis*. Thus by the variation in the sugar moiety, resulted in drastic changes in the bioactivity which implies that the activity depend on the type of the sugar moiety.

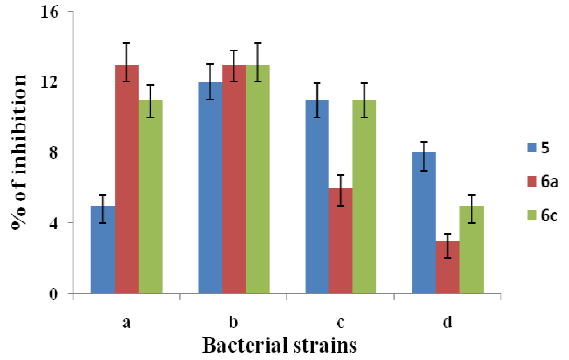


Figure 10 Antibacterial activity of the compounds, **5**, **6a** and **6c** against different bacterial strains (75 µg/mL), (a), *Streptococcus pyogenes*; (b), *Bacillus subtilis*; (c), *Pseudomonas aeruginosa* and (d), *Klebsiella pneumoniae*.

3. Conclusion

In summary, we have synthesized six different unsymmetrical sugar-chalcone based *bis*-triazole derivatives in excellent yield and characterized using different spectral techniques. The existence of β-anomeric form and 1,4-regioisomer of the synthesized sugar-triazole derivatives were confirmed using NMR studies. From the ligand-protein interaction experiment, one mode of binding was observed for chalcone *bis*-triazole derivatives and two mode of binding for bis triazole aldehyde. Thus, in conclusion all the three *bis*-triazole derivatives interacted efficiently with BSA. In particular, the xylose coupled triazole, **6c** found to have strong interaction compared to the other two derivatives, **5** and **6a**. UV titration studies revealed the groove binding nature of the chalcone based *bis*-triazole derivatives with CT-DNA. The interaction of the synthesized sugar-triazole derivatives with BSA found to be better compared to that of CT-DNA and it is well supported from docking analysis. These results could evidently show the strong influence of the triazole based ligands on the bio-molecules and would be useful in designing and developing new triazole based ligands as potential agents capable of interacting at the specific sites of BSA and DNA.

4. Experimental Section

4.1 General Methods

D-Glucose, butyraldehyde, acetyl acetone, 3,4-dihydroxy benzaldehyde, 2,4-dihydroxybenzaldehyde, propargyl bromide were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., USA and were of high purity. Sodium bicarbonate, potassium carbonate, pyrrolidine, zinc chloride, hydrogen bromide in acetic acid, sodium azide, tertiary butanol, copper sulphate, sodium ascorbate were obtained from Loba-chemie. Other reagents, such as hydrochloric acid and solvents (AR Grade) were obtained from Sd-fine, India, in high purity and were used without any purification. Acetic anhydride was purchased from Fischer Chemicals Pvt. Ltd., India. BSA and CT DNA were purchased from Genei, Bangalore, India. The molecular weight of BSA was assumed to be 68000. All BSA solutions were prepared in the Tris-HCl (pH=7.0, 5 mmol/L), 50 mmol/L NaCl buffer and stored in the dark at 4 °C. Absorption spectral titrations were carried out in 50mM Tris HCl-buffer at room temperature to investigate the binding affinity between CT-DNA and ligand. Tetra(n-butyl)ammonium perchlorate (TBAP), used as a supporting electrolyte in the electrochemical measurement was purchased from Fluka and recyrstallized from hot methanol. The solvents were purified according to standard methods. Column chromatography was performed on silica gel (100–200 mesh). NMR spectra were recorded on a Bruker DRX 300 MHz instrument in either $CDCl_3$ or DMSO-d_6. Chemical shifts were referenced to internal TMS. Single crystal XRD studies were carried out using Bruker axs (Kappa apex 2) instrument. Cyclic voltammetry was carried out using CH11008 electrochemical analyzer using a three-electrode cell in which a glassy carbon electrode is the working electrode, a saturated Ag/AgCl electrode is the reference electrode and a platinum wire serves as an auxiliary electrode under oxygen free conditions. The concentration of the ligand is 10^{-3} M. TBAP (10^{-1} M) is used as a supporting electrolyte. Elemental analyses were performed using a Perkin-Elmer 2400 series CHNS/O analyzer. While assigning the spectral data, several abbreviations were used, and these include 'Ar' for aromatic, 'Sac' for saccharide, 'Ace' for acetal and 'trz' for triazole respectively.

4.2 Biological activity assay:

The in vitro antibacterial activity of the triazole derivatives, **5**, **6a** and **6c** were determined by the well diffusion method described by Perez *et al*. Luria Bertani agar was used for the preparation of plates. The medium was poured on to the sterile petri-dishes of 90 mm diameter. The agar was allowed to set at ambient temperature. Fresh bacterial cultures of four human pathogenic bacteria such as *Bacillus subtilis* MTCC121 (Gram

positive), *Klebsiella pneumoniae* MTCC3384 (Gram negative), *Streptococcus pyogenes* MTCC442 (Gram positive) and *Pseudomonas aeruginosa* MTCC424 (Gram negative) were spread as a thin film on the surface of the Luria Bertani agar plates. After incubation using a sterile cork borer, wells were cut form the agar in the plate. The compounds were weighed and dissolved in dimethyl sulfoxide (DMSO 10 mg/mL). Aliquots of 25 µL, 50µL and 75 µL of the test solution were poured in to the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature, and they were incubated at 37 ^{o}C for 24 h. Turbidity was adjusted with sterile broth so as to correspond to 0.5 McFarland standard. Then the plates were examined for any zone of growth inhibition. Inhibition zones were recorded as the diameter of growth-free zones including the diameter of the well in mm at the end of the incubation period. 75µL of chloroform served as the control.

4.3 General Procedure for the synthesis of sugar-chalcones having *bis*-triazole moiety

To a solution of sugar-chalcone (1 equiv.) in THF and water mixture in 1:1 ratio, sugar azide (2.2 equiv.), $CuSO_4$ (0.2 equiv.) and sodium ascorbate (0.4 equiv.) were added. It was then stirred at room temperature for 24 hrs. After the completion of the reaction the solvent was evaporated and it was extracted with $CHCl_3$ (200ml) and water (200 ml). The organic layer was then evaporated, slurried and purified by column chromatography.

4.3.1 3,4-*bis*(prop-2-ynyloxy)benzaldehyde (2a)

Colourless crystal, Yield: 89 %, mp 96-98 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 9.82 (s, 1H, -CHO), 7.51-7.45 (m, 2H, Ar-H), 7.11 (d, J = 8.1 Hz, 1H, Ar-H), 4.79 (d, J = 2.4 Hz, 2H, -OCH_2), 4.76 (d, J = 2.4 Hz, 2H, -OCH_2), 2.51-2.49 (s, 2H, -C≡CH), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 190.7, 152.7, 147.8, 130.8, 126.7, 113.2, 112.6, 77,7, 77.5, 76.7, 76.5, 56.8, 56.7; Anal. Calc. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71, Found: C, 72.82; H, 4.69.

4.3.2 2,4-*bis*(prop-2-ynyloxy)benzaldehyde (2b)

Colourless crystal, Yield: 77 %, mp 104-106 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 10.32 (s, 1H, -CHO), 7.85 (d, J = 9.0 Hz, 1H, Ar-H), 6.70 (s, 1H, Ar-H), 6.67 (d, J = 1.8 Hz, 1H, Ar-H), 4.81 (d, J = 2.4 Hz, 2H, -OCH_2), 4.77 (d, J = 2.4 Hz, 2H, -OCH_2), 2.60 (s, 2H, -C≡CH), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 188.1, 163.6, 161.4, 130.6, 120.1, 107.5, 100.4, 77.5, 77.0, 76.7, 76.5, 56.5, 56.1, Anal. Calc. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71, Found: C, 72.85; H, 4.67.

4.3.3 (*E*)-1-(4,6-*O*-butylidene-β-D-glucopyranosyl)-4-(3,4-*bis*-prop-1'-oxo-2'-ynephenyl)-but-3-ene-2-one (3a)

White solid, Yield: 91 %, mp 156-158 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.55 (d, J = 15.9 Hz, 1H, Alk-H), 7.28 (s, 1H, Ar-H), 7.22 (d, J = 8.4 Hz, 1H, Ar-H), 7.08 (d, J = 8.1 Hz, 1H, Ar-H), 6.68 (d, J = 16.2 Hz, 1H, Alk-H), 4.82 (s, 4H, -OCH_2), 4.55 (t, J = 5.0 Hz, 1H, Sac-H), 4.15 (dd, J = 3.9 Hz, J = 9.6 Hz, 1H, Sac-H),3.98-3.92 (m, 1H, Sac-H), 3.74 (t, J = 8.7 Hz, 1H, Sac-H), 3.44 (t, J = 4.7 Hz, 2H, Sac-H), 3.34 (dd, J = 4.8 Hz, J = 10.2 Hz, 1H, Sac-H), 3.26 (t, J = 9.0 Hz, 1H, Sac-H), 3.17 (s, 1H, Sac-OH), 3.12 (m, 2H, Sac-H), 2.97 (dd, J = 6.9 Hz, J = 16.1 Hz, 1H, Sac-H), 2.57 (s, 2H, -C≡CH), 1.66-1.62 (m, 2H, -CH_2), 1.44 (q, J = 7.5 Hz, 2H, -CH_2), 0.94 (t, J = 7.2 Hz, 3H, -CH_3), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 198.0, 149.9, 147.6, 143.5, 128.3, 125.1, 123.8, 114.2, 113.9, 102.5, 80.5, 78.0, 77.9, 76.4 (2C), 76.2, 75.4, 74.5, 70.6, 68.3, 57.0, 56.7, 43.4, 36.2, 17.5, 13.9, Anal. Calc. for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43, Found: C, 66.35; H, 6.41.

4.3.4 (*E*)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(3,4-*bis*-prop-1'-oxo-2'-yne phenyl)-but-3-ene-2-one (3b)

Syrupy liquid, Yield: 89 %, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.50 (d, J = 16.2 Hz, 1H, Alk-H), 7.27-7.18 (m, 2H, Ar-H), 7.07 (d, J = 8.4 Hz, 1H, Ar-H), 6.63 (d, J = 16.2 Hz, 1H, Alk-H), 5.24 (t, J = 9.3 Hz, 1H, Sac-H), 5.08 (t, J = 9.8 Hz, 1H, Sac-H), 4.99 (t, J = 9.6 Hz, 1H, Sac-H), 4.81 (d, J = 2.4 Hz, 4H, -OCH_2), 4.26 (dd, J = 4.8 Hz, J = 12.6 Hz, 1H, Sac-H), 4.15-4.12 (m, 1H, Sac-H), 4.03 (dd, J = 2.1 Hz, J = 12.3 Hz, 1H, Sac-H), 3.74-3.70 (m, 1H, Sac-H), 3.02 (dd, J = 8.4 Hz, J = 16.2 Hz, 1H, -CH_2), 2.67 (dd, J = 3.2 Hz, J = 16.2 Hz, 1H, -CH_2), 2.58-2.56 (m, 2H, -C≡CH), 2.02 (m, 12H, -$OCOCH_3$), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 196.0, 170.6,170.2, 170.0, 169.6, 149.9, 147.6, 143.4, 128.2, 125.0, 123.8, 114.2, 113.7, 78.0, 77.9, 76.6, 76.4, 75.7, 74.2, 71.7, 68.5, 62.1, 56.9, 56.7, 42.5, 29.7, 20.7 (2C), 20.6 (2C), Anal. Calc. for $C_{30}H_{32}O_{12}$: C, 61.64; H, 5.52, , Found: C, 61.60; H, 5.49.

4.3.5 (*E*)-1-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-(3,4-*bis*-prop-1'-oxo-2'-ynephenyl)-but-3-ene-2-one (3c)

Syrupy liquid, Yield: 88 %, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.50 (d, J = 16.2 Hz, 1H, Alk-H), 7.27 (s, 1H, Ar-H), 7.21 (d, J = 8.4 Hz, 1H, Ar-H), 7.07 (d, J = 8.4 Hz, 1H, Ar-H), 6.63 (d, J = 15.9 Hz, 1H, Alk-H), 5.22 (t, J = 9.5 Hz, 1H, Sac-H), 5.01 (dd, J = 5.7 Hz, J = 10.1 Hz, 1H, Sac-H), 4.93 (t, J = 6.9 Hz, 1H, Sac-H), 4.81 (s, 2H, -OCH_2), 4.80 (s, 2H, -OCH_2), 4.09-4.01 (m, 2H, Sac-H), 3.33 (t, J = 11.0 Hz, 1H, Sac-H), 2.98 (dd, J = 9.0 Hz, J = 16.1 Hz, 1H, Sac-H), 2.63 (dd, J = 3.0 Hz, J = 16.2 Hz, 1H, Sac-H), 2.56 (t, J = 2.3 Hz, 2H, -C≡CH), 2.04 (s, 9H, -$OCOCH_3$), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 196.3, 170.3, 170.1, 169.9, 149.9, 147.6, 143.5, 128.2, 125.1, 123.8, 114.2, 113.8, 78.0, 77.9, 76.5, 76.4, 74.8, 73.7, 72.0, 69.3, 66.8, 56.9, 56.7, 42.4, 20.8, 20.7 (2C), Anal. Calc. for $C_{27}H_{28}O_{10}$: C, 63.28; H, 5.51, Found: C, 63.25; H, 5.47.

4.3.6 (*E*)-1-(4,6-*O*-butylidene-β-D-glucopyranosyl)-4-(2,4-*bis*-prop-1'-oxo-2'-yne phenyl)-but-3-ene-2-one (4a)

Syrupy liquid, Yield: 84 %, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.87 (d, J = 16.2 Hz, 1H, Alk-H), 7.52 (d, J = 8.7 Hz, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 6.72-6.62 (m, 2H, Ar-H, Alk-H), 4.77 (d, J = 2.4 Hz, 2H, -OCH_2), 4.74 (d, J = 2.4 Hz, 2H, -OCH_2), 4.53 (t, J = 5.1 Hz, 1H, Ace-H), 4.14 (dd, J = 4.2 Hz, J = 9.8 Hz, 1H, Sac-H), 3.96-3.89 (m, 1H, Sac-H), 3.73 (t, J = 8.7 Hz, 1H, Sac-H), 3.43 (t, J = 8.9 Hz, 2H, Sac-H), 3.38-3.30 (m, 1H, Sac-H), 3.25 (t, J = 9.0 Hz, 1H, Sac-H), 3.11 (dd, J = 4.2 Hz, J = 16.2 Hz, 1H, -CH_2), 2.99 (dd, J = 6.9 Hz, J = 16.1 Hz, 1H, -CH_2), 2.57 (t, 2.4 Hz, 2H, -C≡CH), 1.65-1.60 (m, 2H, -CH_2), 1.42 (q, J = 7.8 Hz, 2H, -CH_2), 0.92 (t, J = 7.4 Hz, 3H, -CH_3), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 198.6, 160.7, 157.8, 138.7, 130.0, 125.3, 117.7, 107.4, 102.5, 100.8, 80.4, 77.8, 77.2, 76.4, 76.2, 76.1, 75.4, 74.8, 70.6, 68.4, 56.3, 56.0, 43.3, 36.2, 17.5, 13.9, Anal. Calc. for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43, Found: C, 66.33; H, 6.38.

4.3.7 (*E*)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(2,4-*bis*-prop-1'-oxo-2'-ynephenyl)-but-3-ene-2-one (4b)

Syrupy liquid, Yield: 82 %, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.82 (d, J = 16.2 Hz, 1H, Alk-H), 7.52 (d, J = 8.7 Hz, 1H, Ar-

H), 6.75-6.63 (m, 3H, Alk-H, Ar-H), 5.23 (t, J = 9.3 Hz, 1H, Sac-H), 5.08 (t, J = 9.8 Hz, 1H, Sac-H), 4.99 (t, J = 9.8 Hz, 1H, Sac-H), 4.79 (s, 2H, -OCH_2), 4.74 (s, 2H, -OCH_2), 4.27 (dd, J = 4.8 Hz, J = 12.3 Hz, 1H, Sac-H), 4.16-4.00 (m, 2H, Sac-H), 3.72 (dd, J = 3.0 Hz, J = 9.8 Hz, 1H, Sac-H), 3.02 (dd, J = 8.4 Hz, J = 16.2 Hz, 1H, Sac-H), 2.67 (dd, J = 3 Hz, J = 16.2 Hz, 1H, Sac-H), 2.59 (t, J = 2.1 Hz, 2H, -C≡CH), 2.05-2.01 (m, 12H, -$COCH_3$), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 196.5, 170.7, 170.3, 170.0, 169.6, 160.7, 157.8, 138.5, 130.1, 125.2, 117.6, 107.4, 100.8, 77.9, 77.8, 76.5, 76.2, 75.7, 74.3, 71.8, 68.6, 62.1, 60.4, 56.3, 56.0, 42.3, 21.0, 20.7, 20.6, 14.2; Anal. Calc. for $C_{30}H_{32}O_{12}$: C, 61.64; H, 5.52, Found: C, 61.61; H, 5.47.

4.3.8 (*E*)-1-(2,3,4-tri-*O*-acetyl-*β*-D-xylopyranosyl)-4-(2,4-*bis*-prop-1'-oxo-2'-ynephenyl)-but-3-ene-2-one (4c)

Syrupy liquid, Yield: 79 %, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.84(d, J = 16.2 Hz, 1H, Alk-H), 7.53 (d, J = 8.4 Hz, 1H, Ar-H), 6.77-6.64 (m, 3H, Alk-H, Ar-H), 5.23 (t, J = 9.5 Hz, 1H, Sac-H), 5.05-4.92 (m, 2H, Sac-H), 4.80 (d, J = 2.4 Hz, 2H, -OCH_2), 4.75 (d, J = 2.7 Hz, 2H, -OCH_2), 4.10-4.03 (m, 2H, Sac-H), 3.34 (t, J = 11.0 Hz, 1H, Sac-H), 2.99 (dd, J = 9.0 Hz, J = 16.0 Hz, 1H, Sac-H), 2.67 (d, J = 3.0 Hz, 1H, Sac-H), 2.62-2.58 (m, 2H, -C≡CH), 2.05 (s, 9H, -$COCH_3$), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 196.8, 170.3, 170.0, 169.9, 160.7, 157.8, 138.6, 130.1, 125.3, 117.6, 107.4, 100.8, 77.9, 77.8, 76.4, 76.2, 74.9, 73.8, 72.0, 69.3, 66.8, 56.3, 56.0, 42.3, 20.8, 20.7 (2C), Anal. Calc. for $C_{27}H_{28}O_{10}$: C, 63.28; H, 5.51, Found: C, 63.23; H, 5.48.

4.3.9 1-(formyl)-4-{3,4-*bis*[1-(2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-4'-hydroxymethylenetriazolo]}-benzene (5)

Yield: 80 %, mp 202-204ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 9.84 (s, 1H, -CHO), 8.33 (s, 1H, trz-H), 8.18 (s, 1H, trz-H), 7.53-7.50 (m, 2H, Ar-H), 7.12 (d, J = 8.4 Hz, 1H, Ar-H), 6.03 (t, J = 9.8 Hz, 1H, Sac-H), 5.57-5.40 (m, 6H, Sac-H), 5.38 (s, 2H, -OCH_2), 5.35 (s,2H, -OCH_2), 4.45 (t, J = 7.8Hz, 1H, Sac-H), 4.32 (dd, J = 5.1Hz, J = 12.6 Hz, 2H, Sac-H), 4.18-4.07 (m, 2H, Sac-H), 3.43 (t, J = 7.1 Hz, 2H, Sac-H), 2.10-2.01 (m, 24H, -$COCH_3$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 190.7, 176.1, 170.5, 169.9 (2C), 169.6, 169.5, 168.9, 168.8, 153.9, 148.7, 144.4, 144.3, 130.9, 126.6, 122.0, 121.7, 114.8, 114.2, 85.7 (2C), 77.2, 75.0, 72.7, 70.3, 70.2, 67.8, 63.6, 63.3, 61.6, 56.2, 43.9, 31.1, 20.9, 20.6, 20.5 (4C), 20.0, 18.1; **EI-MS**: Calc. for $C_{41}H_{48}N_6O_{21}$,960.29 m/z found 961.29 (M+H); Anal. Calc. for $C_{41}H_{48}N_6O_{21}$: C, 51.25; H, 5.04; N, 8.75, Found: C, 51.20; H, 4.98; N, 8.71.

4.3.10 (*E*)-1-(4,6-*O*-butylidene-*β*-D-glucopyranosyl)-4-{3,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetylglucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (6a)

Pale Yellow solid, Yield: 78 %, mp 132-134 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.33 (s, 1H, trz-H), 8.24 (s, 1H, trz-H), 7.50 (d, J = 15.6 Hz, 1H, Alk-H), 7.26 (s, 1H, Ar-H), 7.17 (d, J = 7.8 Hz, 1H, Ar-H), 7.02 (d, J = 8.4 Hz, 1H, Ar-H), 6.67 (d, J = 15.9 Hz, 1H, Alk-H), 6.04 (t, J = 7.4 Hz, 2H, Sac-H), 5.56-5.33 (m, 8H, Sac-H, -OCH_2), 4.55 (t, J = 5.1 Hz, 1H, Sac-H), 4.34-4.31 (m, 2H, Sac-H), 4.19-4.10 (m, 6H, Sac-H), 3.94 (t, J = 10.5 Hz, 1H, Sac-H), 3.74 (t, J = 8.3 Hz, 1H, Sac-H), 3.40 (t, J = 9.9 Hz, 4H, Sac-H), 3.26 (t, J = 9.0 Hz, 2H, Sac-H), 3.14-3.09 (m, 2H, Sac-H), 2.94 (dd, J = 7.2 Hz, J = 15.5 Hz, 1H, Sac-H), 2.11-2.05 (m, 24H, -$COCH_3$), 1.81-1.80 (m, 2H, -CH_2), 1.43 (q, J = 6.3 Hz, 2H, -CH_2), 1.27 (t, J = 6.9 Hz, 3H, -CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 197.9, 170.6 (2C), 170.0 (2C), 169.6 (2C), 168.9 (2C), 150.9, 148.5, 144.7, 144.5, 143.2, 128.5, 124.9, 124.1, 122.0 (2C), 115.2, 115.0, 102.4, 85.7 (2C), 80.5, 77.2, 76.2, 75.3, 75.0, 74.6, 72.7, 70.6, 70.3, 68.3, 67.8, 63.7, 63.3, 61.7, 60.4, 43.6, 36.2, 31.6, 22.6, 21.1, 20.6 (3C), 20.5, 20.1, 20.0, 17.5, 14.2, 14.1, 13.9; **EI-MS**: Calc. for $C_{54}H_{68}N_6O_{26}$ 1216.42, m/z found 1217.42 (M+H); Anal. Calc. for $C_{54}H_{68}N_6O_{26}$: C, 53.29; H, 5.63; N, 6.90, Found: C, 53.25; H, 5.60; N, 6.86.

4.3.11 (*E*)-1-(2,3,4,6-tetra-*O*-acetylated-*β*-D-glucopyranosyl)-4-{3,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetyl glucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (6b)

White solid, Yield: 65 %, mp 120-122 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.35 (s, 1H, trz-H), 8.23 (s, 1H, trz-H), 7.47 (d, J = 15.9 Hz, 1H, Alk-H), 7.30 (s, 1H, Ar-H), 7.18 (d, J = 8.1 Hz, 1H, Ar-H), 7.03 (d, J = 8.4 Hz, 1H, Ar-H), 6.63 (d, J = 16.2 Hz, 1H, Alk-H), 6.04 (t, J = 9.8 Hz, 2H, Sac-H), 5.57-5.51 (m, 3H, Sac-H), 5.48-5.39 (m, 3H, -OCH_2, Sac-H), 5.35-5.20 (m, 8H, -OCH_2, Sac-H), 5.08 (t, J = 9.8 Hz, 2H, Sac-H), 4.98 (t, J = 9.6 Hz, 1H, Sac-H), 4.34-4.24 (m, 4H, Sac-H), 3.75-3.70 (m, 2H, Sac-H), 3.01 (dd, J = 8.4 Hz, J = 16.5 Hz, 1H, -CH_2), 2.66 (dd, J = 3.0 Hz, J = 16.4Hz, 1H, -CH_2), 2.10-2.02 (m, 36 H, -$COCH_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 195.9, 170.5 (2C), 170.3 (4C), 170.0 (2C), 169.6 (2C), 168.9 (2C), 151.3, 149.7, 144.8 (2C), 143.0, 128.5, 124.9, 124.2, 121.8, 115.4 (2C), 101.8, 85.7, 82.6 (2C), 77.2, 75.7, 75.0, 74.2 (2C), 72.8 (2C), 71.7, 70.3 (2C), 68.5, 67.8, 67.7 (2C), 63.9, 62.0, 61.6 (2C), 42.9, 20.7 (2C), 20.6 (8C), 20.5; Anal. Calc. for $C_{58}H_{70}N_6O_{30}$: C, 52.33; H, 5.30; N, 6.31, Found: C, 52.28; H, 5.26; N, 6.27.

4.3.12 (*E*)-1-(2,3,4-tri-*O*-acetylated-*β*-D-xylopyranosyl)-4-{3,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetylglucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (6c)

White solid, Yield: 73 %, mp 106-108 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.27 (s, 1H, trz-H), 8.15 (s, 1H, trz-H), 7.40 (d, J = 16.2 Hz, 1H, Alk-H), 7.17 (s, 1H, Ar-H), 7.10 (d, J = 9.0 Hz, 1H, Ar-H), 6.95 (d, J = 8.4 Hz, 1H, Ar-H), 6.55 (d, J = 16.2 Hz, 1H, Alk-H), 5.96 (t, J = 10.4 Hz, 2H, Sac-H), 5.48-5.43 (m, 2H, Sac-H), 5.40-5.32 (m, 4H, Sac-H, -OCH_2), 5.26-5.12 (m, 4H, Sac-H), 4.92-4.82 (m, 2H, Sac-H), 4.27-4.23 (m, 2H, Sac-H), 4.11-4.07 (m, 2H, Sac-H), 3.99-3.97 (m, 4H, Sac-H), 3.42 (t, J =6.9 Hz, 1H, Sac-H), 3.33 (t, J = 10.8 Hz, 1H, Sac-H), 2.96 (dd, J = 9.0 Hz, J = 16.2 Hz, 1H, -CH_2), 2.62 (dd, J = 3.0 Hz, J = 15.9 Hz, 1H, -CH_2), 2.02-1.96 (m, 33H, -$COCH_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 196.3, 176.1, 172.0, 170.5, 170.3, 170.0 (2C), 169.9 (2C), 169.5, 168.9, 168.8, 151.0, 148.6, 144.6 (2C), 143.3, 128.4, 125.0, 124.2, 121.9, 121.8, 115.4, 115.1, 85.7, 77.2, 75.0, 74.8, 73.7 (2C), 72.8, 72.0, 70.3 (2C), 69.3, 67.8 (2C), 66.8, 63.8, 63.4, 61.6, 56.2, 43.9, 42.6, 20.9, 20.7 (6C), 20.6 (2C), 20.5, 20.1; **EI-MS**: Calc. for $C_{55}H_{66}N_6O_{28}$,1258.39 m/z found 1259.40 (M+H); Anal. Calc. for $C_{55}H_{66}N_6O_{28}$: C, 52.46; H, 5.28; N, 6.67, Found: C, 52.43; H, 5.23; N, 6.62.

4.3.13 (*E*)-1-(4,6-*O*-butylidene-*β*-D-glucopyranosyl)-4-{2,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetyl glucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (7a)

Pale yellow solid, Yield: 67 %, mp 146-148 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.12 (s, 1H, trz-H), 8.04 (s, 1H, trz-H), 7.87

(d, J = 16.1 Hz, 1H, Alk-H), 7.50 (d, J = 8.4 Hz, 1H, Ar-H), 6.72-6.61 (m, 3H, Ar-H, Alk-H), 5.99-5.90 (m, 2H, Sac-H), 5.50-5.41 (m, 2H, Sac-H), 5.32-5.26 (m, 4H, Sac-H), 4.77-4.66 (m, 4H, -OCH_2), 4.54 (bs, 2H, Sac-OH), 4.35-4.29 (m, 2H, Sac-H), 4.18-4.06 (m, 4H, Sac-H), 3.77-3.66 (m, 2H, Sac-H), 3.43-3.21 (m, 6H, Sac-H), 2.89-2.79 (m, 2H, -CH_2), 2.09-2.05 (m, 24H, -$COCH_3$), 1.63-1.62 (m, 2H, -CH_2), 1.42 (q, J = 6.6 Hz, 2H, -CH_2), 1.27 (t, J = 7.1 Hz, 3H, -CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 198.7, 170.6, 170.5, 170.0, 169.6 (2C), 169.4, 169.1, 169.0, 157.8, 138.6, 136.0, 135.4, 132.8, 132.2, 130.5, 130.2, 125.2, 121.6, 121.2, 117.6, 117.3, 102.4 (2C), 85.8, 80.5, 77.2, 76.1, 75.7, 75.3, 75.2, 72.6, 70.6, 70.3, 68.3, 67.7, 62.0, 61.5, 60.4, 56.0, 36.2 (2C), 33.9, 25.3, 24.4, 21.0, 20.7 (2C), 20.5 (2C), 20.1, 17.5, 14.2, 13.9; Anal. Calc. for $C_{54}H_{68}N_6O_{26}$: C, 53.29; H, 5.63; N, 6.90, Found: C, 53.26; H, 5.59; N, 6.86.

4.3.14 (*E*)-1-(2,3,4,6-tetra-*O*-acetylated-β-D-glucopyranosyl)-4-{2,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetylglucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (7b)

White solid, Yield: 61 %, mp 126-128 ºC, **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.02 (s, 1H, trz-H), 7.99 (s, 1H, trz-H), 7.84 (d, J = 16.2 Hz, 1H, Alk-H), 7.53 (d, J = 8.4 Hz, 1H, Ar-H), 6.67-6.54 (m, 3H, Alk-H, Ar-H), 5.89 (t, J = 8.9 Hz, 1H, Sac-H), 5.42 (t, J = 6.0 Hz, 1H, Sac-H), 5.37 (t, J = 4.4 Hz, 2H, Sac-H), 5.25 (s, 2H, -OCH_2), 5.19-5.11 (m, 6H, -OCH_2, Sac-H), 4.92 (t, J = 10.7 Hz, 1H, Sac-H), 4.26-4.17 (m, 4H, Sac-H), 4.11-3.64 (m, 8H, Sac-H), 2.98-2.90 (m, 1H, -CH_2), 2.63-2.50 (m, 1H, -CH_2), 2.01-1.94 (m, 36H, -$COCH_3$), **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 196.4, 170.7, 170.5, 170.3, 170.0 (2C), 169.9, 169.6, 169.4 (2C), 169.3, 168.9, 168.8, 161.5, 161.3, 158.5, 157.9, 144.2, 144.1, 138.4, 130.2, 130.1, 124.8, 121.7, 121.2, 117.4, 117.1, 108.2, 100.6, 100.0, 85.8 (2C), 77.2, 75.7, 75.3, 75.2, 74.3, 72.6, 71.8, 70.3, 68.6, 67.7, 62.2, 61.5 (2C), 42.4, 31.6, 29.7 (2C), 22.6, 20.8, 20.6 (4C), 20.5, 20.1,14.1; Anal. Calc. for $C_{58}H_{70}N_6O_{30}$: C, 52.33; H, 5.30; N, 6.31, Found: C, 52.29; H, 5.28; N, 6.28.

4.3.15 (*E*)-1-(2,3,4-tri-*O*-acetylated-β-D-xylopyranosyl)-4-{2,4-*bis*[1'-(2',3',4',6'-tetra-*O*-acetylglucopyranosyl)-4'-hydroxymethylenetriazolo]phenyl}but-3-ene-2-one (7c)

White solid, Yield: 64 %, mp 110-112 ºC, **^{1}H NMR** (300 MHz, CDCl3): δ 8.09 (s, 1H, trz-H), 8.07 (s,1H, trz-H), 7.84 (d, J = 16.3Hz, 1H, Alk-H), 7.52 (d, J= 8.7 Hz, 1H, Ar-H), 6.76-6.62 (m, 3H, Alk-H, Ar-H), 5.97 (t, J = 9.3 Hz, 1H, Sac-H), 5.47-5.43 (m, 4H, Sac-H), 5.34-5.27 (m, 4H, Sac-H), 5.04-4.92 (m, 6H, Sac-H, -OCH_2), 4.79 (d, J = 2.1 Hz, 2H, -OCH_2), 4.36-4.04 (m, 6H, Sac-H), 3.35 (t, J = 11.0 Hz, 1H, Sac-H), 3.01-2.93 (m, 1H, -CH_2), 2.72-2.66 (m, 1H, -CH_2), 2.10-2.05 (m, 33H, -$COCH_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) : δ 196.7, 170.5 (2C), 170.3, 170.1, 170.0 (2C), 169.4 (2C), 169.3, 168.9 (2C), 158.5, 144.2, 144.1, 138.5, 130.1, 125.2, 121.7, 121.2, 117.3, 108.2, 100.4, 85.8 (2C), 75.3, 75.2, 74.9, 73.8, 72.6 (2C), 72.0 (2C), 70.3 (2C), 69.4 (2C), 67.7 (2C), 66.7 (2C), 61.6 (2C), 42.3, 20.8 (2C), 20.7 (6C), 20.6, 20.5, 20.1; Anal. Calc. for $C_{55}H_{66}N_6O_{28}$: C, 52.46; H, 5.28; N, 6.67, Found: C, 52.43; H, 5.25; N, 6.63.

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Supplementary data

A copy of 1H, 13C NMR, DEPT-45, DEPT-135, APT, COSY spectrum, mass spectrum, Ksv graphs, photo copies of antibacterial studies can be found in supplementary data. Crystallographic data for the crystal structure in this paper has been deposited in Cambridge Crystallographic Data Centre as supplementary publication number 824908 and 841652 for compounds 2a and 2b respectively.

Notes and references

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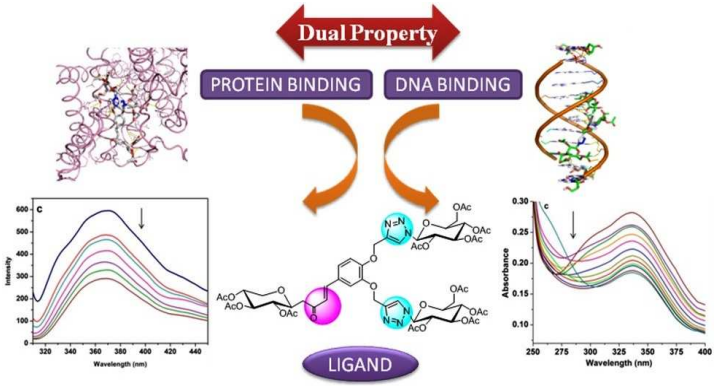
Graphical abstract



Bis-triazole derivatives accomplished by [3+2] cycloaddition methodology were well characterized. Interaction of bis-triazoles with BSA and CT-DNA had good correlation with experimental and docking studies. Compounds showed moderate to excellent antibacterial activity.