



SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL EVALUATION AND DOCKING STUDY OF NEW CHALCONE DERIVATIVES CONTAINING 1, 3, 5- TRIAZINANE-1, 3, 5-TRIYL) MOIETY

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ABSTRACT

This research depicted the synthesis of new chalcone derivatives containing 1,3,5-triazinane-1,3,5-triyl) moiety. The first step included the cyclocondensation of 4-aminoacetophenone with aqueous formaldehyde in ethanol at room temperature to afford 1, 3, 5-triacetophenone-1, 3, 5-hexahydro-s-triazine as starting material. Treatment of triazine derivative with different substituted aromatic aldehydes afforded the novel chalcone derivatives. The synthesized compounds were characterized by FT-IR and ¹H NMR spectroscopy. The antimicrobial activity against several bacterial species as well as against *Candida albicans* was evaluated. Docking study of the synthesized compounds against glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents was achieved to explore and explain the interactions of the discovered hits with in the binding pocket of the target enzyme. The docking results enhanced the activity of new derivatives as promising antimicrobial agents.

Key words: Triazine, Chalcone, Antimicrobial.

INTRODUCTION

Chalcone derivatives have important application in medicinal field. Recently, several articles dealing with Chalcone compounds reveals significant attention to these derivatives due to their diversified therapeutic activities like anticancer, antiviral, antifungal, anti-malarial and anti-bacterial activities¹⁻⁵. These facts motivated us to synthesis novel chalcone derivatives containing 1,3,5-triazinane-1,3,5-triyl) moiety and illustrated their activities against some gram positive and gram negative bacteria as well as *Candida albicans*, the most common fungi species. Autodock 4.2, the effective autodock tool was

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used to study the binding affinity of the synthesized chalcones (**2-9**) inside the binding pocket of glucosamine-6-phosphatesynthase.

EXPERIMENTAL

Synthetic part

Melting points were determined in open capillary tubes and are uncorrected. ¹H NMR spectra (solvent DMSO-d₆ or CDCl₃) were recorded on Bruker DMX-500 spectrophotometer 300 MHz or 500 MHz spectrometer with TMS as internal standard. FTIR spectra were recorded directly in a SHIMADZU FT-IR 8400S, Bruker spectrophotometer.

General procedure of the synthesis of 1, 3, 5-triaryl-1, 3, 5-hexahydro-s-triazines (**1**)⁶

To a mixture of 4-aminoacetophenone (0.01 mol) dissolved in (20 mL) absolute ethanol, aqueous formaldehyde solution (37%, 6 mL) was added with stirring at room temperature for 0.5 hr, the mixture was left standing at room temperature overnight. The formed precipitate was filtered, washed with hot ethanol, and the filtrate was poured into crushed ice to obtain the product. Purification by recrystallization (in EtOH) gave 1, 3, 5-triaryl-1, 3, 5-hexahydro-s-triazines (85%) as yellow crystal. m.p. 180-182°C; IR (cm⁻¹): 3084 ν(C-H) Ar 1656 ν(C=O), 1593 ν(C=C) Ar, 1315 ν(C-N); ¹H NMR: 2.4 ppm (s, 9H, CH₃), 4.6 ppm (s, 3H, N-CH-N), 6.7 ppm (d, 6H, Ar-H), 7.3 ppm (s, 3H, (N-CH-N), 7.7 ppm (d, 6H, Ar-H).

General procedure of synthesis of chalcones (**2-8**)⁷

To a mixture of 1, 3, 5-triaryl-1, 3, 5-hexahydro-s-triazines (**1**), (0.001 mole) and substituted aryl aldehydes (0.003 mol), aqueous solution of NaOH (40%, 6 mL) was added with stirring. The reaction mixture was kept three days at room temperature, then poured into crushed ice and acidified with diluted HCl (10%). The solid separated was recrystallized from suitable solvent to obtain the target chalcones (**2-8**). Table 1 shows the physical properties and the yields, while Table 2 reveals the IR and ¹H NMR data of the synthesized compounds.

Antimicrobial activity

Newly synthesized compounds (**2-9**) were tested for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus SPP* (gram+ve), *Escherichia coli*, *Klebsiella pneumonia* (gram -ve), as well as *C. albicans* using the dilution method. DMSO was run as a control and the test was performed at 10⁻⁵ M, using DMSO solvent. Each experiment was made in triplicate and the average reading was taken⁷.

Table 1: Physical properties for compounds b (1-8)

Compd. No.	m.p. (°C)	Color	Solvent of recrystallization	Molecular formula	M. wt.	Yield (%)
2	150-152	Yellow	EtOH 90%	C ₄₈ H ₃₉ N ₃ O ₃	705	78
3	158-160	Yellow	DMF 60%	C ₄₈ H ₃₆ Br ₃ N ₃ O ₃	942.5	70
4	240-243	Light-orange	EtOH 95%	C ₄₈ H ₃₆ Br ₃ N ₃ O ₃	942.5	75
5	149-151	Yellow	EtOH 90%	C ₄₈ H ₃₆ Cl ₃ N ₃ O ₃	807	67
6	221-223	Orange	EtOH abs	C ₄₈ H ₃₉ N ₆ O ₉	840.8	60
7	226-228	orange	EtOH 85%	C ₄₈ H ₃₆ N ₆ O ₉	840.8	69
8	135-137	Yellow	2-Propanol	C ₅₁ H ₄₅ N ₃ O ₆	795.9	73
9	140-142	Orange	Methanol	C ₅₄ H ₅₄ N ₆ O ₃	835	77

Table 2: FTIR Spectral data for compounds (2-9)

Compd. No.	$\nu(\text{C-H})$ Ar cm ⁻¹	$\nu(\text{C-H})$ Alp cm ⁻¹	$\nu(\text{C=O})$ cm ⁻¹	$\nu(\text{C=C})$ Ar cm ⁻¹	$\nu(\text{Others})$ cm ⁻¹	¹ H NMR
2	3082	2880	1653	1608	H	-
3	3030	2958	1651	1604	P-Br = 669	-
4	3061	2949	1649	1608	O-Br = 661	-
5	3030	2890	1658, 1649	1591	P-Cl = 779	-
6	3084	2943	1651	1591	P-NO ₂ = 1516, 1338	-
7	3076	2960	1651	1591	O-NO ₂ = 1523, 1346	-
8	3028	2953	1651	1595	P-OCH ₃ = 812	3.7 ppm (s, 9H, -OCH ₃), 4.6 ppm (s 6H, (N-CH ₂ N)), 6.7 ppm (d, 12H, Ar-H), 7.5 ppm (d, 6H (CH = CH) alkene) 7.7 ppm (d, 12H, Ar-H)

Cont...

Compd. No.	$\nu(\text{C-H})$ Ar cm^{-1}	$\nu(\text{C-H})$ Alp cm^{-1}	$\nu(\text{C=O})$ cm^{-1}	$\nu(\text{C=C})$ Ar cm^{-1}	$\nu(\text{Others})$ cm^{-1}	$^1\text{H NMR}$
9	3053	2935	1653	1570	P-N(CH ₃) ₂ = 813	2.4 ppm (s, 18H, -N (CH ₃) ₂), 4.6 ppm (s, 6H, (N-CH ₂ -N)), 6.7 ppm (d, 12H, Ar-H), 7.6 ppm (d, 6H, (CH = CH) alkene), 7.9 ppm (d, 12H, Ar-H)

Docking study

In this study, we used Auto Dock 4.2 package software to investigate the affinity of synthesized to the binding pocket of GlcN-6-P synthase. The pdb file format of enzyme as receptor was obtained from the RCSB Protein Data Bank (PDB code 1 MOQ) and used as a rigid molecule. Water molecules were removed and hydrogen atoms were added to the protein amino acids. All the docked compounds were drawn using Chem Draw ultra 7.0 as mole file and the energies of compounds were minimized then converted into the pdb format using open Babel 2.3.1 software. During the docking, the grid dimensions were 60·60·60 Å with points separated by 0.375 Å. The X, Y and Z coordinates were specified as 31.0, 17.0 and -2.0, respectively. Lamarckian Genetic Algorithm was employed as the docking algorithm with 10 runs, 150 population sizes, 2,500,000 maximum numbers of energy evaluations and 27,000 maximum numbers of generations⁷.

RESULTS AND DISCUSSION

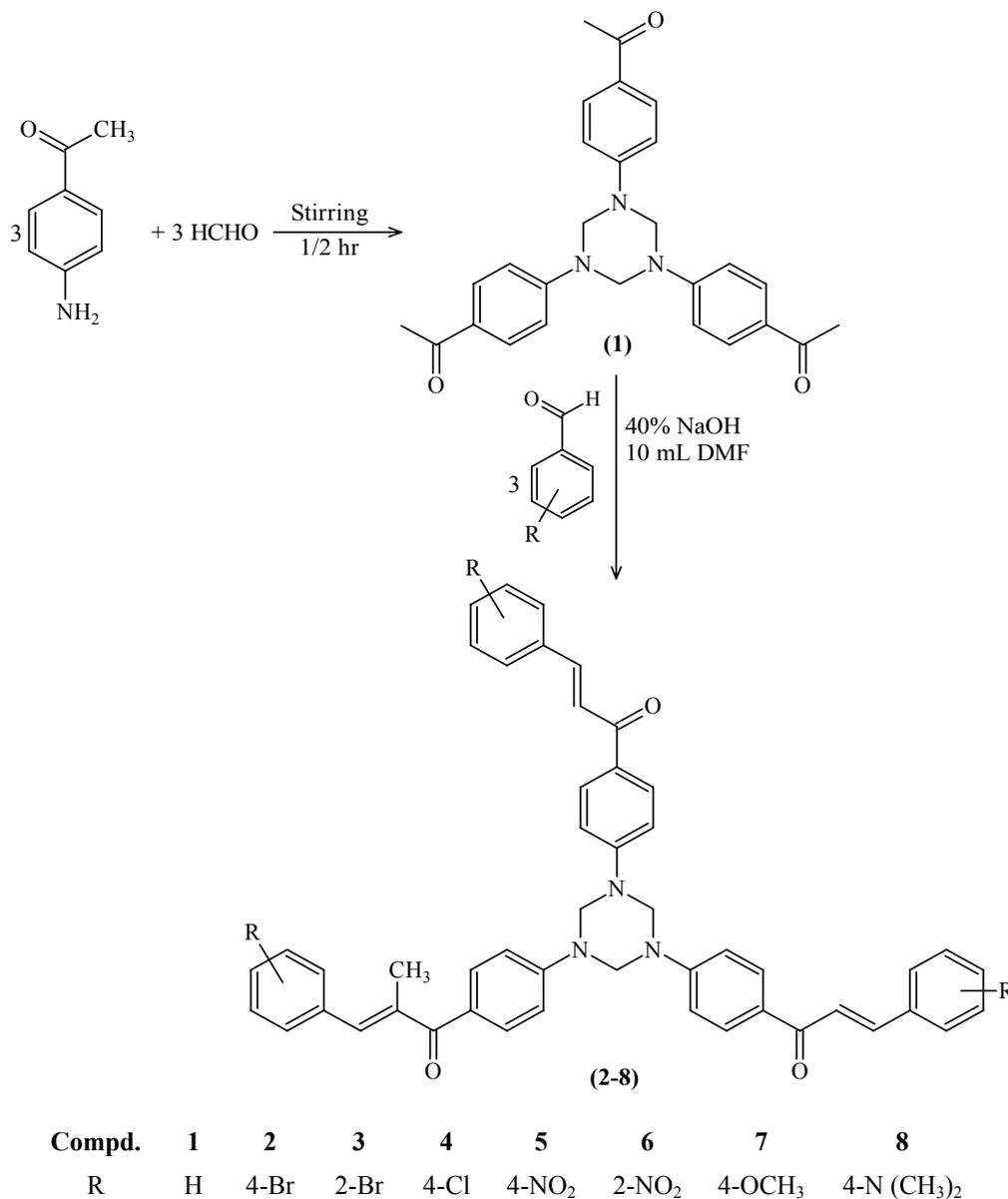
Synthesis of 1, 3, 5-triacetophenone-1, 3, 5-hexahydro-s-triazine (1)

1, 3, 5-triacetophenone-1, 3, 5-hexahydro-s-triazine was synthesized by the cyclocondensation of 4-aminoacetophenone with aqueous formaldehyde (37%) in absolute ethanol as shown in **Scheme 1**. The structure of the synthesized compound (**1**) was confirmed by IR and $^1\text{H NMR}$ spectrum. FTIR spectra show the disappearance of stretching bands for (NH₂) group. The spectra also show the appearance of the stretching bands at frequency (1656) cm^{-1} related to (C=O) group. $^1\text{H-NMR}$ (Fig. 1) of compound **1** strongly enhanced the identification of structure.

Synthesis of chalcone derivatives (2-9)

Chalcones derivatives (**2-9**) were prepared by treatment of starting material **1** with different substituted aromatic aldehydes in DMF in presence of 40% NaOH (**Scheme 1**).

The synthesized compounds were characterized by FTIR and ^1H NMR spectrum. Fig. 2 shows the FTIR spectrum of compound **5**, which indicated the appearance of (C=O) stretching frequency at 1658 and 1649 cm^{-1} with the stretching frequency of (C=C) at (1591) cm^{-1} . ^1H NMR spectrum of compounds **8** and **9** illustrated in Figs. 3 and 4, respectively.



Scheme 1: Synthesis of chalcones derivatives

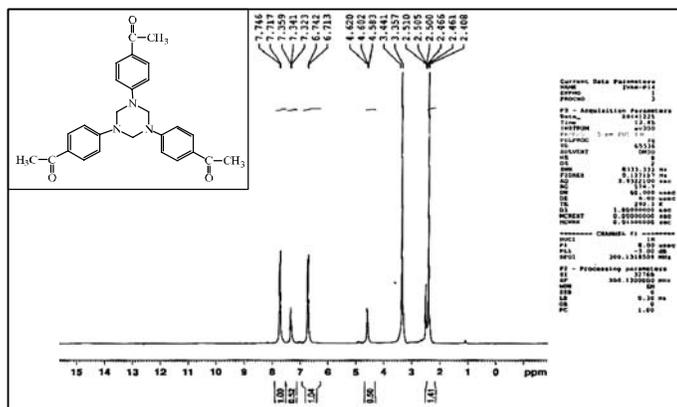


Fig. 1: ¹H-NMR spectrum of compound 1

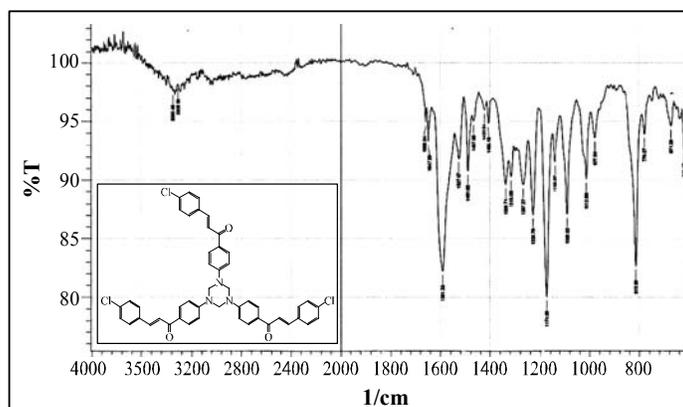


Fig. 2: FTIR spectrum of compound 5

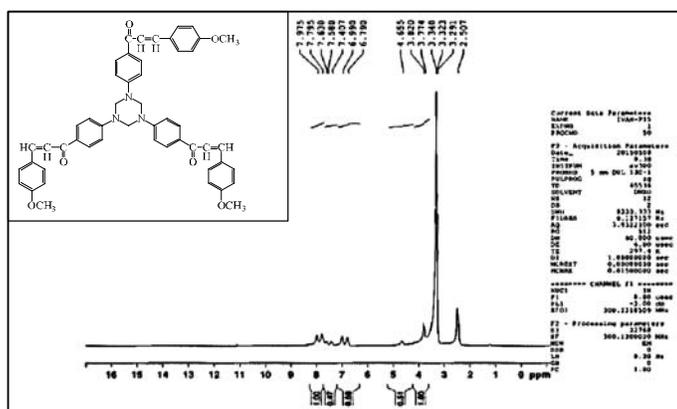


Fig. 3: ¹H-NMR spectrum of compound 8

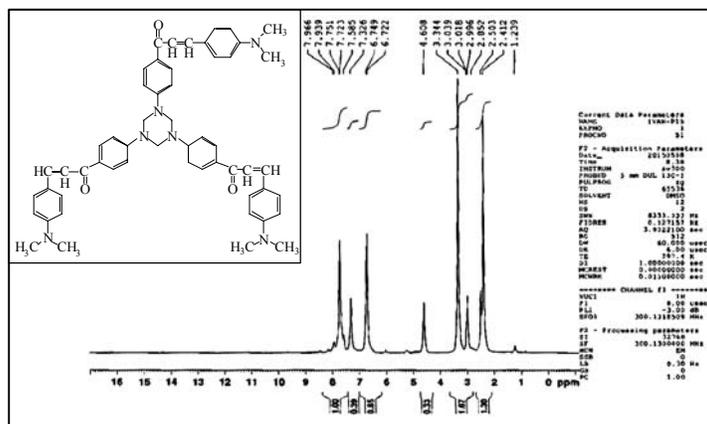


Fig. 4: ^1H -NMR spectrum of compound 9

Biological evaluation

Chalcone compounds **2-9** were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Streptococcus* SPP (gram + ve), *Escherichia coli*, *Klebsiellapneumoniae* (gram-ve), as well as *C. albicans* using the dilution method. Table 3 shows the antibacterial and antifungal activities of the synthesized derivatives at 10^{-5} M concentration.

Table 3: Antibacterial activity data of some of the synthesized compounds

Compd. No.	Conc.	Gram positive		Gram negative		Fungal
		<i>Staphylococcus S. aureus</i>	<i>Streptococcus sp</i>	<i>E. coli</i>	<i>Klbe pneumniae</i>	<i>Candida</i>
2	10^{-5}	-	11 mm	10 mm	-	8 mm
3	10^{-5}	-	-	-	-	-
4	10^{-5}	-	-	-	-	-
5	10^{-5}	9 mm	-	10 mm	9 mm	14 mm
6	10^{-5}	9 mm	9 mm	16 mm	-	-
7	10^{-5}	-	-	-	8 mm	-
8	10^{-5}	-	-	-	8 mm	-
9	10^{-5}	-	9 mm	-	-	-

Docking study

The potent activity of synthesized compounds (2-8) as new antimicrobial agents, prompted us to study the docking of these derivatives inside the active site of glucose amine-6-phosphate synthase, the potential target for antibacterial and anti-fungal agents. X-ray study of glucosamine-6-phosphate synthase with divergent inhibitors shows that the binding pocket of the enzyme include the following residues, Cys 300, Gly 301, Thr 302, Ser 303, Ser 347, Gln 348, Ser 349, Thr 352, Val 399, Ser 401, Ala 602 and Lys 603 as shown in Fig. 5, which illustrates the insilico active pocket prediction of amino acid residues in binding with glucose amine-6-phosphate enzyme obtained from PDB sum⁸.

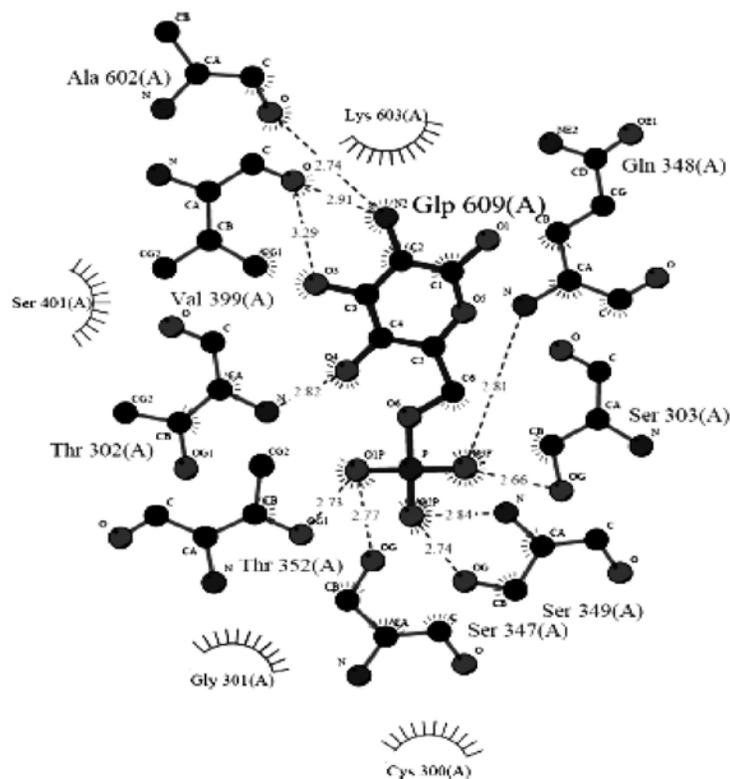
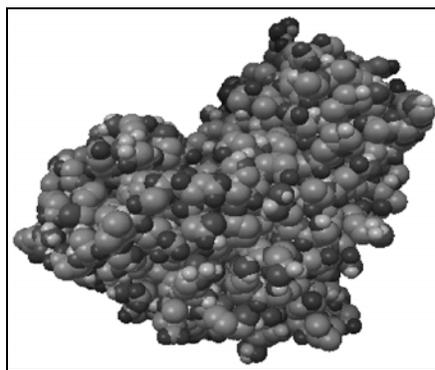
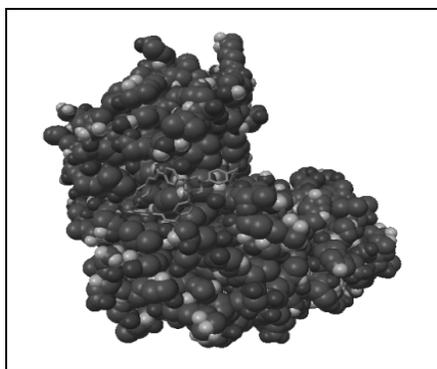
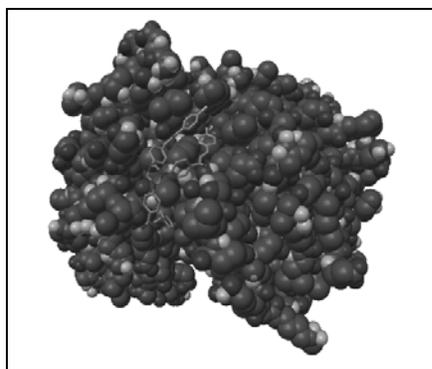


Fig. 5: Lig plot of Glc N-6-P showing the binding of glucosamine-6-phosphate in an active site of enzyme

Docking studies are computational techniques for the exploration of the possible binding modes of a ligand to a given receptor, enzyme or other binding site. In this study, we used auto dock 4.2 m to evaluate the binding energy of ligands inside the known 3 D structure of target enzyme. Auto Dock 4.2 software consists of two main programs, auto grid

that pre-calculates grid maps of inter action energies for various atom types of ligand with a macromolecule and auto dock, which performs the docking of the ligand to specified grids⁹. For the typical systems, docking is carried out using a Lamarckian Genetic Algorithm (LGA), it is run several times to give several docked conformations (ten conformers by default) ranking according to their binding and intermolecular energies. Several parameters were also predicted by the auto-dock program such as inhibition constant, number of hydrogen bonds and others. Fig. 6 illustrates the binding of the best generated conformers for compounds b(1-8) inside the binding pocket of target enzyme. Table 2 indicates the molecular docking parameters of compounds b (1-8). The high ranking binding energies of the generated conformer were -7.37 , -7.62 and -7.61 kcalmol⁻¹ for b(1-8), respectively. The high docking energies of all generated conformers of compound 2b are strongly proportional to the antibacterial activities as shown in Table 1. Inhibition constant K_i , intermolecular energy and hydrogen bonds were also determined and depicted in Table 2. Fig. 4 1-8 binding of the high ranking generated conformers for compounds H_a (1-8), respectively inside the binding pocket of GlcN-6-p synthase. (1) 3D structure of GlcN-6-synthase.

**(b1)****(b1)****(b2)**

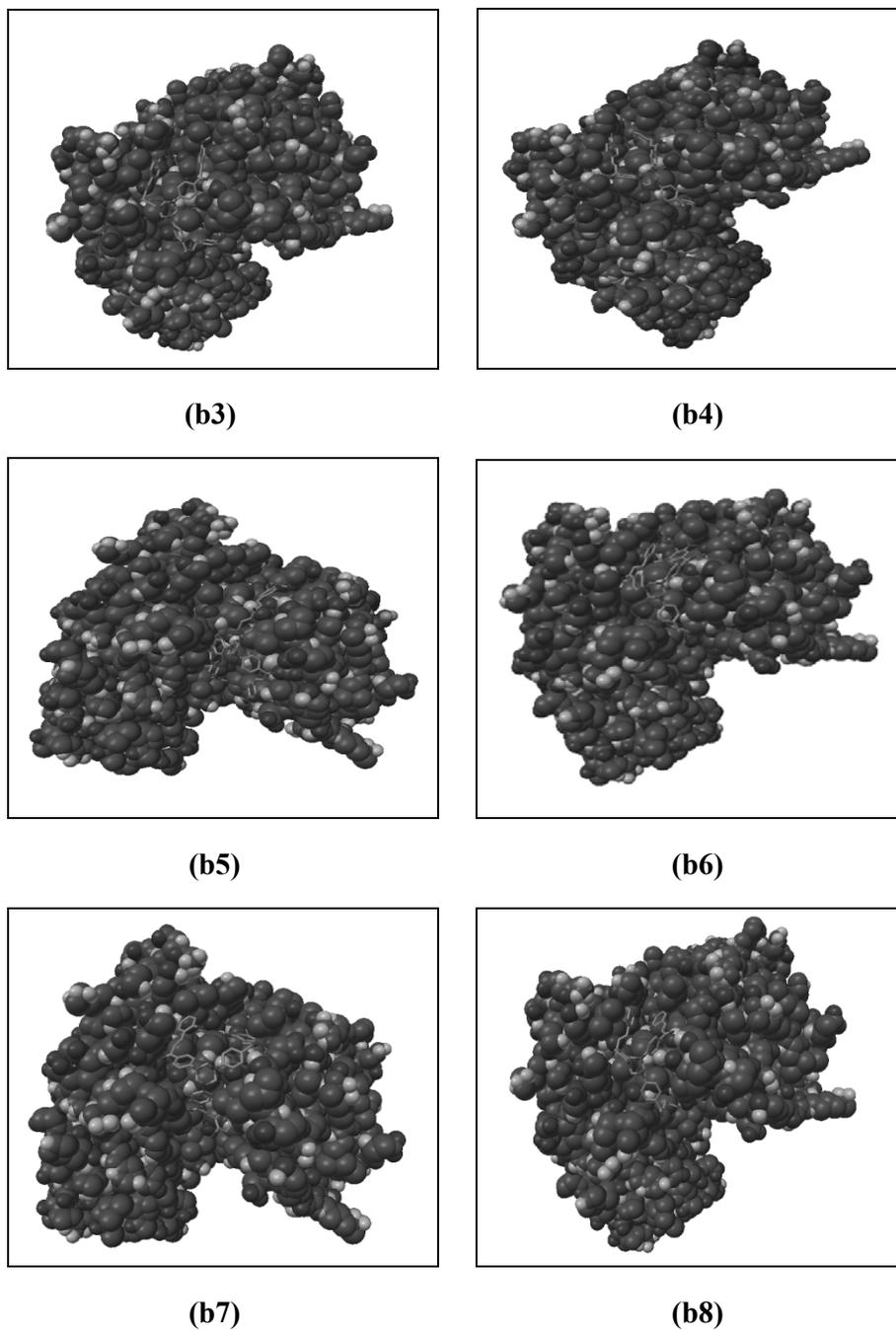


Fig. 6: 3D structure of GlcN-6-synthase binding of the high ranking generated conformers for compounds b(1-8), respectively inside the binding pocket of GlcN-6-p synthase

Table 4: Molecular docking parameter of chalcone derivatives with glucosamine-6-phosphate synthase

Compd. a	Binding energy (kcal mol ⁻¹)	Inhibition constant	Intermolecular
		(mM), (μM)* (nM)**	(kcal mol ⁻¹)
Compd. 1			
1	-4.73	340.42*	-9.21
2	-5.35	120.51*	-9.82
3	-7.35	4.12*	-11.82
4	-7.28	4.59*	-11.76
5	-7.66	2.43*	-12.13
6	-6.19	29.11*	-10.66
7	-2.45	16.09*	-6.92
8	-5.65	72.74*	-10.12
9	-7.65	2.49*	-12.12
10	-4.49	512.91*	-8.96
Compd. 2			
1	-6.6	14.52*	-10.18
2	-4.72	344.38*	-8.3
3	-6.03	37.99*	-9.61
4	-7.16	5.66*	-10.74
5	-5.79	57.02*	-9.37
6	-5.15	169.13*	-8.73
7	-5.36	116.86*	-8.94
8	-4.88	265.47*	-8.46
9	-7.1	6.21*	-10.68
10	-6.13	31.93*	-9.71

Cont...

Compd. a	Binding energy (kcal mol ⁻¹)	Inhibition constant	Intermolecular
		(mM), (μM)* (nM)**	(kcal mol ⁻¹)
Compd. 3			
1	-3.47	2.88	-7.94
2	-1.34	103.53	-5.82
3	-1.52	77.39	-5.99
4	0.31	-	-4.16
5	-5.65	72.00*	-10.13
6	-2.36	18.77	-6.83
7	-5.45	100.95*	-9.93
8	-2.52	14.22	-6.99
9	0.75	-	-3.72
10	1.45	-	-3.02
Compd. 4			
1	-8.69	425.91**	-12.27
2	-6.85	9.59*	-10.43
3	-7.3	4.49*	-10.88
4	-8.67	443.88**	-12.25
5	-7.02	7.12*	-10.6
6	-6.06	36.34*	-9.64
7	-5.18	158.59*	-8.76
8	-7.69	2.29*	-11.27
9	-5.5	93.53*	-9.08
10	-6.97	7.81*	-10.55
Compd. 5			
1	-3.02	6.11	-7.5
2	-2.42	16.85	-6.89

Cont...

Compd. a	Binding energy (kcal mol ⁻¹)	Inhibition constant	Intermolecular
		(mM), (μM)* (nM)**	(kcal mol ⁻¹)
3	-4.52	487.52*	-8.99
4	-3.2	4.51	-7.67
5	1.04	-	-3.43
6	-4.36	637.91*	-8.83
7	-2.62	11.97	-7.1
8	-3.2	4.51	-7.67
9	+1.01 x 10 ³	-	+1.01x10 ³
10	-4.03	1.11	-8.51
Compd. 6			
1	-5.24	144.18*	-9.71
2	-6.3	24.02*	-10.78
3	-7.3	4.46*	-11.77
4	-5.85	51.14*	-10.33
5	-7.81	1.89*	-12.28
6	-6.62	14.03*	-11.09
7	-7.74	2.11*	-12.22
8	-6.68	12.60*	-11.16
9	-3.2	4.5	-7.68
10	-7.4	3.76*	-11.87
Compd. 7			
1	-5.45	100.37*	-9.03
2	-5.15	169.03*	-8.73
3	-6.02	38.73*	-9.6
4	-4.86	274.83*	-8.44
5	-5.66	71.28*	-9.24
6	-6.15	31.17*	-9.73

Cont...

Compd. a	Binding energy (kcal mol ⁻¹)	Inhibition constant	Intermolecular
		(mM), (μM)* (nM)**	(kcal mol ⁻¹)
7	-5.26	140.21*	-8.84
8	-5.84	51.99*	-9.42
9	-5.79	56.88*	-9.37
10	-7.03	6.98*	-10.61
Compd. 8			
1	-4.38	614.45*	-7.96
2	-7.49	3.22*	-11.07
3	-6.95	8.05*	-10.53
4	-4.7	359.11*	-8.28
5	-8.03	1.30*	-11.61
6	-5.48	96.44*	-9.06
7	-6.85	9.53*	-10.43
8	-7.26	4.79*	-10.84
9	-6.95	8.00*	-10.53
10	-5.88	48.86*	-9.46

CONCLUSION

New chalcones were synthesized (**2-8**) and characterized using IR, and ¹H NMR spectroscopy method. The antimicrobial study of the derivatives against some gram-positive and gram negative species as well as against *candida albicans* was studied using the well diffusion method. The novel chalcones were identified as promising antimicrobial agents. To explain the activity of new derivatives, we explore the binding affinity of the compounds against glucosamine-6-phosphatesynthase, the target enzyme for the antimicrobial agents. Docking study strongly enhanced the activity of these compounds as new discovered hits.

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