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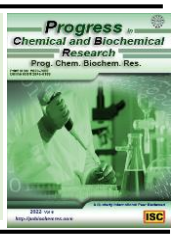

Original Research Article

Chalcone Derivatives Containing Imine Moieties as New Antibacterial Agents: Synthesis, *SAR*, Anti-biofilm Activity and Docking study

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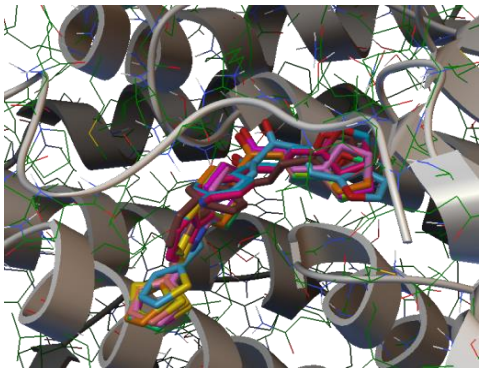
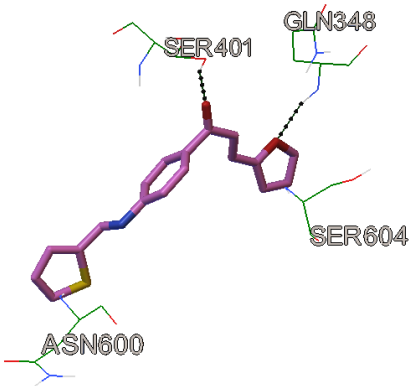
ABSTRACT

A new series of 3-furan-2-yl-1-*p*-aryl-propenone derivatives containing imine moieties (**1-7**) were synthesized and characterized using spectral analysis. The synthesized derivatives were screened *in vitro* against several bacterial species, including *Acinetobacter baumannii, Klebsiella pneumonia, Pseudomonas aeruginosa (*Gram-negative bacteria), and *Staphylococcus aureus* (Gram-positive bacteria) to study the effect of different imine moieties on the activity of (*E*)-1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one, which represent the potent hit against different bacterial species. The synthesized compounds were found to exhibit modest to vigorous activity, especially compounds **1**, **4**, and **6-7**. The minimum inhibitory concentrations (MICs) of compound **1** and **6** against *Acinetobacter baumannii and Staphylococcus aureus* were determined. The anti-biofilm activity of the potent discovered compounds (1, 4, 6, and 7) against *Acinetobacter baumannii and Staphylococcus aureus* were also determined. Docking study of the best discovered hits against the active site of glucosamine-6-phosphate synthase, the antimicrobial target enzyme was achieved to explore the interactions of the synthesized hits inside the enzyme residues.

GRAPHICAL ABSTRACT



All the generated conformers for compound **1** inside the active site of enzyme



The 1st generated conformer of derivative **1** inside the active site of enzyme

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1. Introduction

α,β-Unsaturated ketones containing a 1,3-diarylprop-2-en-1-one moiety, known as chalcones and their derivatives have been intensive interest [1-2] due to their versatile activities such as antibacterial [3], antifungal [4-5], antimalarial [6-7], anti-HIV [8], and anticancer [9-11]. Recent studies have been focused on the antimicrobial activities of chalcone derivatives to explore novel skeleton of potent antimicrobial agents in order to control bacterial infection, inhibit the antibiotic-resistant, and prevent the formation of biofilms and other virulence factors in pathogenic bacteria [12]. Several studies have been demonstrated the antibacterial activity of chalcone derivatives against *Staphylococcus aureus* that causes a severe infections such as pneumonia, osteomyelitis, abscess, and meningitis [13-14]. The antibacterial activity of chalcone derivatives were also investigated against *Escherichia coli* and the study exhibited that the chalcone derivatives treated the gastrointestinal tract infections caused by several *E. coli* species in animals and humans [15]. According to Ferraz *et al.* [16] and our previous publication [17], (E)-1-(4-Aminophenyl)-3-(furan-2-yl)prop-2-en-1-one (Figure 1) exhibited potent activity against multi-resistant strains of bacterial species (*+ve* and – *ve* species) as well as *candida albicans*. Therefore, this work aimed to synthesize novel 3-furan-2-yl-1-p-aryl-propenone containing imine moieties (1-7) by modifying the structure of the amino group of target chalcone with different imine substituents. The activity of the new derivatives was tested against several bacterial species. Among the discovered hits, compounds 1, 4, 6, and 7 exhibited promising results against many bacterial species, and the anti-biofilm activities of these derivatives were evaluated. To explore the binding modes of the potent derivatives against the active site of glucosamine-6-phosphate synthase, the target enzyme in antimicrobial chemotherapy, AutoDock 4.2 package tool was used [18]. The docking outcomes illustrate that the active hits bind the docking site in similar manner to that of glucosamine-6-phosphate, the substrate of the target enzyme.

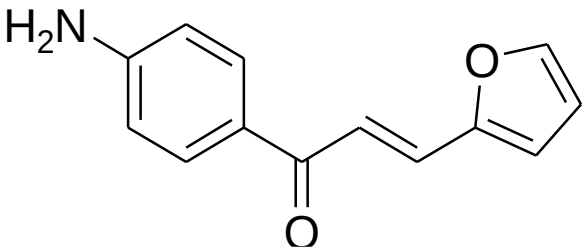


Figure 1. Structure of starting material.

2. Results and Discussion

2.1. Synthesis

The chalcone derivative, (*E*)-1-(4-aminophenyl)-3-furan-2-yl-propenone was synthesized and characterized using a modified protocol as described in our previous work [19]. The *E* configuration of the synthesized derivatives was confirmed form the ^{1}H-NMR spectrum of starting material. The coupling constant value (*j* = 15.3 Hz) of the vinylic protons strongly confirmed the trans-isomer (see 3.1.1). The reaction between the chalcone and the corresponding aldehydes in acidic ethanolic solution afforded the target derivatives (1-7), as displayed in Scheme 1. Structural elucidation of the obtained compounds was achieved by spectral technique. The infra-red spectra of compounds 1-7 reveal stretching bands at 1662-1647 cm^{-1}, 1626-1600 cm^{-1}, and 1600-1593 cm^{-1} regions related to C=O, CH=N and CH=CH groups, respectively. The stretching frequencies disappearance of amino group strongly enhances the elucidation of synthesized compounds. The mass spectra were consistent with the molecular ion peak values of the synthesized derivatives.

The nuclear magnetic resonance spectroscopy strongly confirms the structure elucidation of the synthesized derivatives. The ^{1}H-NMR spectra of compounds 3 and 4 showed singles within the 8.47 and 8.86 ppm regions due to imine protons with the absence of a singlet signal at 6.18 related to the amino group in the starting material. For more information about the spectral data, see the experimental section. The ^{1}H-NMR spectra of the synthesized derivatives were depicted in the ***Supplementary Materials***.

2.2. Antibacterial activity

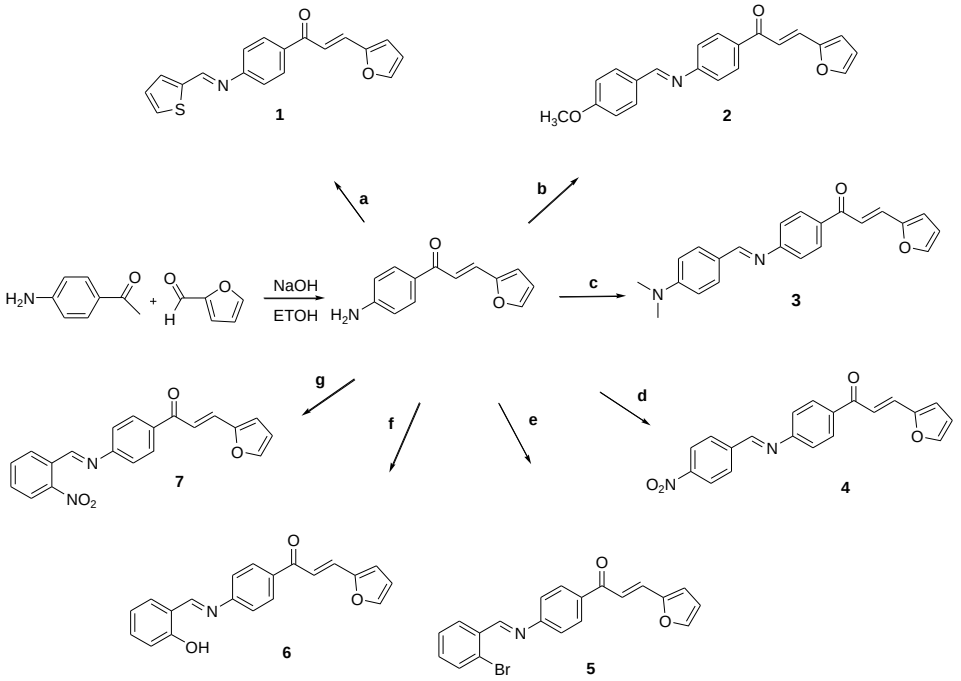
The newly synthesized compounds were screened for their antibacterial activity using a well diffusion method against *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* as gram-negative species and *Staphylococcus aureus* as a gram- positive strain (Table 1). The standard antibiotic ampicillin, was used as a control. In general, *S. aureus* and *A. baumannii* were found to be more susceptible to the tested compounds than the other species of bacteria. The structure activity relationship (SAR) of the synthesized derivatives were examined to compare the effect different imine moieties on the activity of (*E*)-1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one.

The target chalcone was previously synthesized by our team as well as by other groups and published as potent antimicrobial agent [16-17]. The present study illustrated the effect of amino group $-NH_2$ on the antibacterial activity. The converting of this group to versatile imine moieties –N=CHAr afforded chalcone derivatives with good to fear activity. The chalcone scaffold with thiophene-imine moiety (**1**) was more powerful antimicrobial agents than other derivatives, which means that the free NH_2 group not essential for the activity. The chalcone derivatives with para-substituted phenyl-imine moieties, electron-donating groups (**2-3**) exhibit any activity, while the electron-withdrawing group as in compound 4 reveals good imbibition against all the bacterial species.

The electron-releasing group in ortho position enhance the activity as in compound **6** comparing with withdrawing groups in the same position (compound **5** and **7**). The minimum inhibitory concentrations of compound **1** was 0.002 µg/mL against *Acinetobacter baumannii* and *Staphylococcus aureus*, while for compounds 6 was 0.008 µg/mL for the same species. In the present study, *P. aeruginosa* exhibited resistance to the synthesized compounds (**2-3**, **5, 7**). The results of these studies are summarized in Table 1.

2.3. Anti-biofilm activity

The complex matrix of polysaccharides, proteins and DNAs known as bacterial bio-film is responsible for many chronic infections, including cystic fibrosis, osteomyelitis, and dental caries. Several bacterial species acquire resistance to antibiotics by the formation of biofilms, which represent quorum sensing (QS) to coordinate gene expression [20]. Exploring small molecules that inhibit QS activity and prevent bacterial communication represents a powerful strategy to control biofilm formation [21]. The current research explored chalcones incorporating imine moieties with potent antibacterial activities (**1**, **4**, **6**, and **7**) as new scaffolds for anti-biofilm agents. The percent inhibition of biofilms against *Acinetobacter baumannii* and *Staphylococcus aureus* was measured, and the results are indicated in Table 2. The novel compound **4** exhibited a potent effect on bacterial biofilm formation in *Staphylococcus aureus* and was still effective at low concentrations. The coating of the polystyrene surface with compound **4** reduces biofilm formation by 66%. The results show that synthesized compound **4** represents a good chemical skeleton for the enhancement of novel anti-biofilm derivatives. The anti-biofilm activities of the tested compound are summarized in Table 2.



Scheme 1. (a) thiophene-2-carbaldehyde, (b) 4-methoxybenzaldehyde, (c) 4-N,N-dimethylbenzylaldehyde, (d) *p*-nitrobenzaldehyde, (e) 2-bromobenzaldehyde, (f) 2-hydroxybenzaldehyde, and (g) *o*-nitrobenzaldehyde. Solvent: EtOH with two drops of glacial CH_3COOH

Table 1. *In vitro* antimicrobial inhibition zone (mm) of the synthesized compounds.

Compounds	Inhibition zones (mm) at 0.01 mg/mL, 0.001 mg/mL ()*, and MIC () μg/ml **			
	Acinetobacter baumannii	Klebsiella pneumoniae	Pseudomonas aeruginosa	Staphylococcus aureus
1	20, (16)*, (0.002) **	-	12, (-)*	20, (20)*, (0.002) **
2	-	-	-	-
3	-	-	-	-
4	19, (17)*	15, (15)*	12, (-)*	22, (21)*
5	15, (18)*	-	-	18, (15)*
6	17, (13)*, (0.008) **	18, (-)*	13, (-)*	20, (18)*, (0.008) **
7	22, (22)*	13, (-)*	-	15, *
Ampicillin	12	10	10	12

( )* at 0.001 mg/mL - The diameter of the inhibition area is less than 10 mm

2.4. Docking Study

The recent research including the docking study of the potent discovered hits (ligands) inside the active site of glucosamine-6-phosphate synthase to explore binding affinity of the discovered derivatives inside the enzyme cavity.

Table 2. Inhibition percentages of biofilm formation of selected synthesized compounds.

Bacterial isolates	% Inhibition of biofilm formation			
	Compound **1**	Compound **4**	Compound **6**	Compound **7**
Acinetobacter baumannii	30	48	34	48
Staphylococcus aureus	30	66	44	40

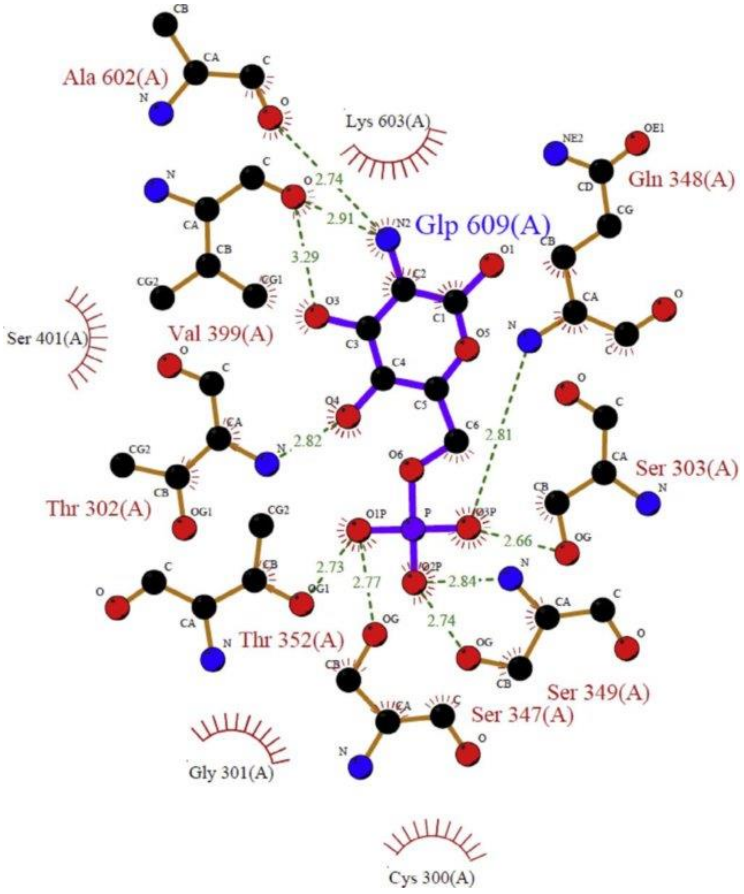


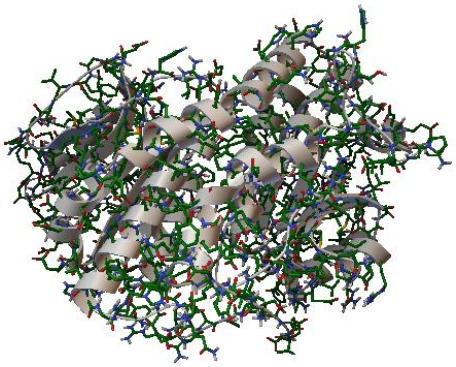
Figure 2. Glucosamine-6-phosphate inside the active site residues of GlcN-6-P.

Table 3. Docking parameters of potent derivatives (**1**, **4**, **6**, and **7**).

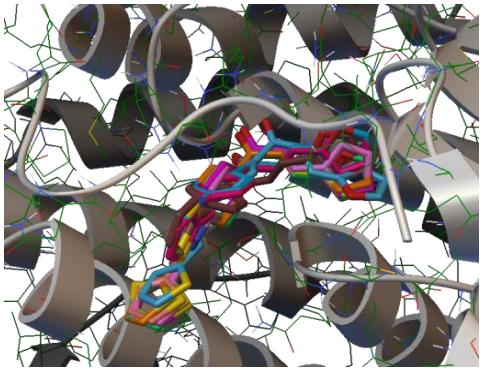
Compounds		Binding Energy (kcal.mol^{-1})	Inhibition constant (μM)	Intermolecular energy (kcal.mol^{-1})	H-bonds	Bonding
1	1	-7.03	7.01	- 8.52	2	GLN348:HN:LIGAND:O SER401:HG:LIGAND:O
	2	-6.99	7.56	- 8.48	1	SER401:HG:LIGAND:O
	3	-6.99	7.51	- 8.84	2	SER401:HG:LIGAND:O GLN348:HN:LIGAND:O
	4	-6.97	7.80	- 8.46	1	SER401:HG:LIGAND:O
	5	-6.79	10.47	- 8.29	1	ALA602:HN:LIGAND:N
	6	-6.79	10.48	- 8.29	1	SER401:HG:LIGAND:O
	7	-6.69	12.56	- 8.18	1	ALA602:HN:LIGAND:N

	8	-6.67	12.98	- 8.16	1	SER401:HG:LIGAND:O
	9	-6.61	14.33	- 8.10	2	SER401:HN:LIGAND:O ALA602:HN:LIGAND:N
	10	-6.23	27.07	- 7.72	2	LIGAND:N:GLU488:OE2 LYS603:HZ3:LIGAND:O
4	1	-6.87	9.28	- 8.66	2	GLN348:HN:LIGAND:O SER349:HN:LIGAND:O
	2	-6.76	11.07	- 8.55	3	SER303:HG:LIGAND:O SER349:HN:LIGAND:O GLN348:HN:LIGAND:O
	3	-6.72	11.79	- 8.51	3	SER303:HG:LIGAND:O SER349:HN:LIGAND:O GLN348:HN:LIGAND:O
	4	-6.65	13.26	- 8.44	2	GLN348:HE21:LIGAND:O SER349:HN:LIGAND:O
	5	-6.39	20.79	- 8.18	2	SER401:HN: LIGAND:O ALA602:HN:LIGAND:N
	6	-6.35	22.27	- 8.14	2	SER401:HN: LIGAND:O ALA602:HN:LIGAND:N
	7	-6.29	24.53	- 8.08	1	ALA602:HN:LIGAND:N
	8	-6.28	25.03	- 8.07	2	ALA602:HN:LIGAND:N SER401:HN: LIGAND:O
	9	-6.27	25.27	- 8.06	3	ALA602:HN:LIGAND:N SER401:HG: LIGAND:O GLN348:HN:LIGAND:O
	10	-6.23	27.10	- 8.02	2	THR302:HN:LIGAND:O ALA602:HN:LIGAND:N
6	1	-7.10	6.30	- 8.88	2	SER401:HG: LIGAND:O ALA602:HN:LIGAND:O
	2	-7.00	7.34	- 8.79	4	ALA602:HN:LIGAND:O LIGAND:H: ALA602:O SER349:HN:LIGAND:O THR302:HN:LIGAND:O
	3	-6.87	9.27	- 8.66	2	SER401:HG: LIGAND:O LYS603:HZ3:LIGAND:O
	4	-6.76	11.03	- 8.55	3	SER349:HN:LIGAND:O THR302:HN:LIGAND:O LIGAND:H: ASP354:OD2
	5	-6.74	11.39	- 8.53	3	ALA602:HN:LIGAND:O GLN348:HN:LIGAND:O THR302:HN:LIGAND:O
	6	-6.72	11.78	- 8.51	3	ALA602:HN:LIGAND:O GLN348:HN:LIGAND:O THR302:HN:LIGAND:O
	7	-6.63	13.89	- 8.42	2	THR352:HG1:LIGAND:O ALA602:HN:LIGAND:O
	8	-6.46	18.35	- 8.25	3	SER401:HG:LIGAND:O ALA602:HN:LIGAND:O GLN348:HN:LIGAND:O
	9	-6.45	18.76	- 8.24	3	THR352:HG1:LIGAND:O ALA602:HN:LIGAND:O THR302:HN:LIGAND:O
	10	-6.23	27.06	- 8.02	2	GLN348:HN:LIGAND:O SER401:HG:LIGAND:O
7	1	-6.71	12.08	- 8.50	2	SER401:HG:LIGAND:O GLN348:HN:LIGAND:O
	2	-6.65	13.40	- 8.44	3	SER401:HN:LIGAND:O LYS603:HZ3:LIGAND:O SER401:HG:LIGAND:O
	3	-6.60	14.57	- 8.39	3	THR302:HN:LIGAND:O SER349:HN:LIGAND:O ALA602:HN:LIGAND:O
	4	-6.56	15.66	- 8.35	2	SER401:HN:LIGAND:O ALA602:HN:LIGAND:N
	5	-6.56	15.48	- 8.35	3	THR302:HN:LIGAND:O SER349:HN:LIGAND:O ALA602:HN:LIGAND:O
	6	-6.51	16.92	- 8.30	3	GLY301:HN:LIGAND:O THR302:HN:LIGAND:O

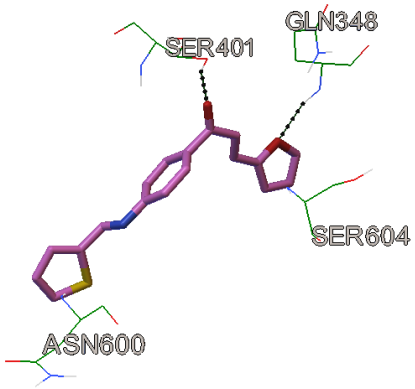
7	-6.49	17.58	- 8.28	2	SER349:HN:LIGAND:O SER401:HN:LIGAND:O SER401:HG:LIGAND:O
8	-6.40	20.42	- 8.19	3	SER401:HN:LIGAND:O LYS603:HZ3:LIGAND:O SER401:HG:LIGAND:O
9	-6.31	23.81	- 8.10	2	THR352:HG1:LIGAND:O ALA602:HN:LIGAND:O
10	-6.12	32.64	- 7.91	1	GLN348:HN:LIGAND:O



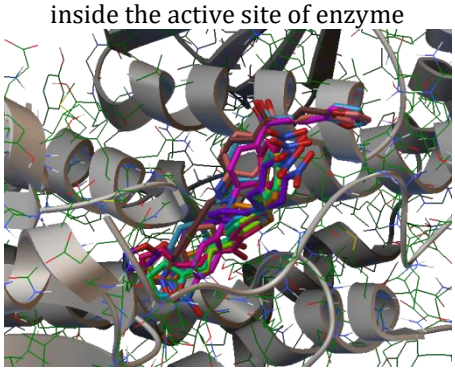
Three dimensional array of GlcN-6-P



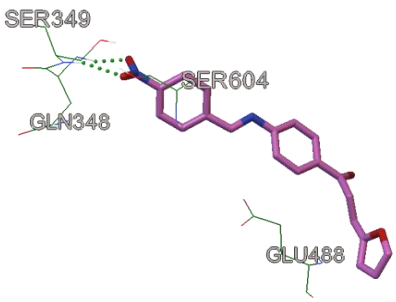
All the generated conformers for compound **1** inside the active site of enzyme



The 1st generated conformer of derivative **1** inside the active site of enzyme

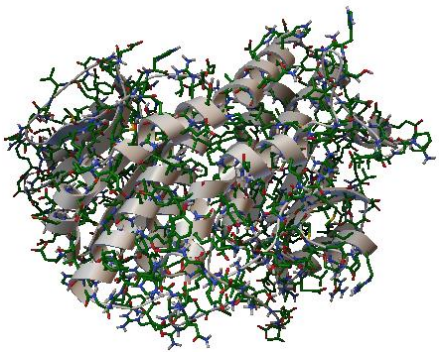


All the generated conformers for compound **4** inside the active site of enzyme

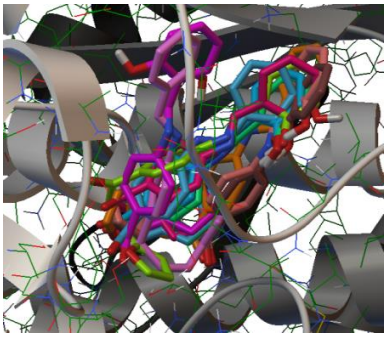


The 1st generated conformer of derivative **4** inside the active site of enzyme

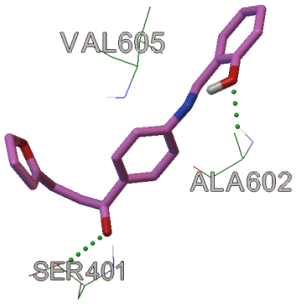
Figure 3. The best conformers of compound **1**, **4**, **6**, and **7** within binding cavity of GlcN-6-P.



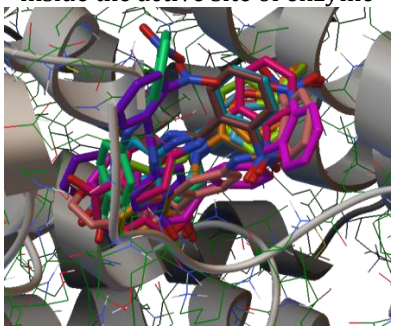
Three dimensional array of GlcN-6-P



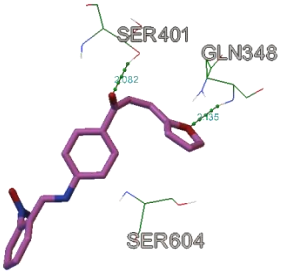
All the generated conformers for compound **6** inside the active site of enzyme



The 1st generated conformer of derivative **6** inside the active site of enzyme



All the generated conformers for compound **7** inside the active site of enzyme



The 1st generated conformer of derivative **7** inside the active site of enzyme

Figure 3. Continued.

The active site residues of target enzyme with the corresponding substrate (glucosamine-6-phosphate) were characterized by X-ray crystallography and illustrated in Figure 2 [22]. Autodock 4.2 bundle software tool was used to evaluate binding of the active derivatives (**1**, **4**, **6**, and **7**) within the enzyme cavity. As a default, ten conformers were generated for each derivative according to their binding energies inside the enzyme active site. The docking outcome parameters for the active hits are listed in Table 3. The best generated conformer for compound **1** fits the active site with -7.03 kcal.mol^{-1}. The predicted inhibition constant was 7.01 μM, while the intermolecular energy was - 8.52 kcal.mol^{-1}. As illustrated in Figure 3, the conformer 1 binds the enzyme residue with two hydrogen bonds. The first hydrogen bond was between oxygen of furan ring and the hydrogen of glutamine residue (GLN348), while the second was between the carbonyl oxygen and the serine residue (SER401). The best binding affinity, as indicated by docking approach was between the chalcone derivative **6** and the enzyme active site. Binding

energy of the high ranking conformer of compound **6** was -7.10 kcal.mol-1 with intermolecular energy equal to -8.88 kcal.mol^{-1}. The best conformer binds the proper enzyme residues (SER401 and ALA602) with two hydrogen bonds, as depicted in Figure 3. The docking study reveals that the interaction between potent derivatives and enzyme residues was directly related to the antimicrobial activity and was considered as an effective tool for activity estimation.

3. Materials and Methods

3.1. Synthesis

All starting materials and solvents were commercially available without further purification and were purchased from Sigma-Aldrich. FT-IR measurements were obtained on an FTIR-8400S Shimadzu system (Tokyo, Japan). ^{1}H-NMR spectra were recorded with an ultra-shielded 300 MHz Bruker spectrophotometer (Berlin, Germany) in DMSO-d6 solution with TMS as the internal standard. Mass spectra were recorded on a GCMS-QP2010 Shimadzu Ultra apparatus (Tokyo, Japan). Uncorrected melting points were determined on an electro-thermal capillary apparatus (Berlin, Germany). The optical density was measured using Elisa reader instrument (BioTek ELX800, USA). The ^{1}H-NMR spectra of the synthesized derivatives uploaded in the ***Supplementary Materials***.

3.1.1. Synthesis of (E)-1-(4-Amino-phenyl)-3-furan-2-yl-propenone

The target compound was prepared following the protocol depicted in a reported reference [23]. Sodium hydroxide (40 %, 1 mL) was added to a solution of 4-aminoacetophenone (1 mmol) in ethanol (96%, 10 mL) and the crude material was stirred for 30 minutes. Then, furan-2-carbaldehyde (1 mmol) was added, and the crude reaction was stirred for 3 hours. The precipitated solid collected after allowing the mixture to stand at room temperature was dried and further recrystallized with EtOH (96%).

Yellow powder, yield 90 %, m.p. 106-108 °C; IR (cm^{-1}): 3441, 3352 (*NH_2*), 3039 (*C-H* Ar), 1633 (*C=O*), 1600 (*C=C*), 1579 (*C=C Ar*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 6.18 *(s, 2H, NH_2), 6.60 (d, J=8.6 Hz, 2H, C-H Ar), 6.66-6.65(m,1H, C-H furan),7.00 (d, J=3.3 Hz, 1H, C-H furan), 7.43 (d, j = 15.3 Hz, 1H, C-H chalcone), 7.51 (d, j = 15.3 Hz, 1H, C-H chalcone), 7.82 (d, J=8.6 Hz, 2H, C-H Ar), 7.86 (d, j= 3.3Hz, 1H, C-H furan). Mass (NCI)* m/e: 213 M^+ *For $C_{13}H_{11}N\ O_2$. R_f=0.5 (2:1,* V/V, *Hexane: Ethyl acetate).*

3.1.2. Synthesis of imine derivatives (1-7)

Schiff bases were synthesized according to a protocol described in a published reference [24]. To a solution of the corresponding aldehyde (1 mmol) in absolute ethanol (4 mL) with a few drops of glacial acetic acid, a chalcone derivative (1 mmol) was added. The crude material was refluxed for 10-12 hours, and the reaction completion was checked by thin layer chromatography using ethyl acetate: hexane as the eluent system (1:2, V/V). The precipitate was collected and washed with ethanol, dried, and then purified by recrystallization from ethanol (96%).

*(E)-3-Furan-2-yl-1-{4-[(thiophen-2-ylmethylene)-amino]-phenyl}-propenone (**1**)*

Yellow powder, yield 70 %, m.p. 80-82 °C; *IR* (cm^{-1}): 3001 (*C-H Ar*), 2929 (*C-H* aliphatic), 1649 (*C=O*), 1616 (*C=N*), 1600 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 6.77 (d, j=3.3, 1H, C-H furan), 6.26 (t, j=3.8, 1H, C-H thiophene), 7.01 (d, j 3.2 Hz, 1 H, C-H furan),7.13 (d, j = 3.3 Hz, 1H, C-H thiophen), 7.38 (d, j = 8.3 Hz, 2H, C-H Ar), 7.54-7.59 (s, 2H, CH=chalcone, C-H furan),7.93-7.81 (m, 2H, CH= chalcone, C-H thiophen), 8.14 (d, j = 8.3 Hz, 2H, C-H Ar), 8.86 (s, 1H, CH=N). *Mass (NCI)* m/e: 307 M^+ *For $C_{18}H_{13}NO_2$* S. *R_f=0.77 (1:2,* V/V, *Hexane:Ethyl acetate).*

*(E)-3-Furan-2-yl-1-{4-[(4-methoxy-benzylidene)-amino]-phenyl}-propenone (**2**)*

Yellow powder, yield 65%, m.p. 115-117 °C; *IR* (cm^{-1}): 3003 (*C-H Ar*), 2968 (*C-H* aliphatic), 1656 (*C=O*), 1624 (*C=N*), 1600 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.85 (s, 3H, *CH_3)*, *6.70-6.71 (m, 1H, C-H furan), 7.11-7.13 (m, 3H, 2 C-H Ar, C-H furan),* 7.35 (d, j = 8.4 Hz, 2H, C-H Ar), *7.55-7.65 (m, 2H, CH= chalcone, C-H furan), 7.92-7.94 (m, 3H, 2H, C-H Ar, C=H chalcone),* 8.13 (d, j = 8.4 Hz, *2H, CH Ar)* 8.59 (s, 1H, CH=N). *Mass (NCI)* m/e: 331 M^+ *For $C_{21}H_{17}NO_3$. R_f=0.77 (1:2,* V/V, *Hexane: Ethyl acetate).*

*(E)-1-{4-[(4-Dimethylamino-benzylidene)-amino]-phenyl}-3-furan-2-yl-propenone (**3**)*

Reddish brown powder, yield 70%, m.p. 174-175 °C; *IR* (cm^{-1}): 3043 (*C-H Ar*), 2895 (*C-H* aliphatic), 1651 (*C=O*), 1622 (*C=N*), 1599 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.03 (s, *6H, 2CH_3*), *6.68-6.70 (m, 1H, C-H furan),* 6.80 (d, j = 8.3 Hz, 2H, C-H Ar), 7.12 (d, j = 2.6 Hz, 1H, *C-H furan*), 7.31 (d, j = 8.1 Hz, 2H, C-H Ar), *7.53-7.64 (m, 2H, C-H furan, CH= chalcone),* 7.78 (d, j = 8.3 Hz, 2H, C-H Ar), *7.92 (s, 1H, CH= chalcone)* 8.11 (d, j = 8.1 Hz, 2H, C-H Ar), 8.47 (s, 1H, CH=N). *Mass (NCI)* m/e: 344 M^+ *For $C_{22}H_{20}N_2O_2$. R_f=0.77 (1:2,* V/V, *Hexane: Ethyl acetate).*

*(E)-3-Furan-2-yl-1-{4-[(4-nitro-benzylidene)-amino]-phenyl}-propenone (**4**)*

Yellow off-white powder, yield 60%, m.p. 158-160 °C; *IR* (cm^{-1}): 3039 (*C-H Ar*), 2976 (*C-H* aliphatic), 1654 (*C=O*), 1600 (*C=N*), 1593 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): *6.71-6.72 (m, 1H, C-H furan), 7.13 (d, j = 3.3 Hz, 1H, C-H furan),* 7.46 (d, j = 8.3 Hz, 2H, C-H Ar), *7.58-7.65 (m, 2H, C-H furan, CH=chalcone),7.94 (s, 1H, CH= chalcone),* 8.17 (d, j = 8.3 Hz, 2H, C-H Ar), 8.32 (d, j = 8.3 Hz, 2H, C-H Ar), 8.42 (d, j = 8.3 Hz, 2H, C-H Ar), 8.86 (s, *1H, CH=N*). *Mass (NCI)* m/e: 246 M^+ *For $C_{20}H_{14}N_2O_4$. R_f=0.71 (1:2,* V/V, *Hexane: Ethyl acetate).*

*(E)-1-{4-[(2-Bromo-benzylidene)-amino]-phenyl}-3-Furan-2-yl-propenone (**5**)*

Yellow off-white powder, yield 65%, m.p. 116-118 °C; *IR* (cm^{-1}): 3041 (*C-H Ar*), 2920 (*C-H* aliphatic), 1662 (*C=O*), 1622 (*C=N*), 1600 (*C=C*), 750 (*C-Br)*. ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 6.70 (m, *1H, C-H furan*), 7.13 (d, *J = 3.1 Hz, 1H, C-H furan*),7.4 (d, j = 8.1 Hz, 2H, C-H Ar), *7.50-7.70 (m, 5H, 3C-H Ar, C-H furan, CH=chalcone),7.79 (d, j= 6.9 Hz, 1H, C-H Ar), 7.94 (s, 1H, CH= chalcone),* 8.16 (d, j = 8.1 Hz, 2H, C-H Ar), 8.81 (s, *1H, CH=N*). *Mass (NCI)* m/e: 380 M^+ *For $C_{20}H_{14}BrNO_2$. R_f=0.84 (1:2,* V/V, *Hexane: Ethyl acetate).*

*(E)-3-Furan-2-yl-1-{4-[(2-hydroxy-benzylidene)-amino]-phenyl}-propenone (**6**)*

Yellow off-white powder, yield 66%, m.p. 170-172 °C; *IR* (cm^{-1}): 3200 (*OH*) 3063 (*C-H Ar*), 2987 (*C-H* aliphatic), 1654 (*C=O*), 1620 (*C=N*), 1599 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm):6.72-6.71 (m, 1H, *C-H* furan), 7.03-6.98 (m, 3H, 2 C-H Ar, C-H furan), 7.14 (d, j = 3.3 Hz, 1H, C-H furan), 7.45 (t, j = 7.0 Hz,1H, C-H Ar), 7.61-7.54 (m, 3H, 2C-H Ar, CH= chalcone), 7.72 (d, j = 7.0, 1H, C-H Ar), 7.94 (s, 1H, CH= chalcone), 8.18-8.47 (d, j = 8.4 Hz, 2H, C-H Ar), 9.04 (s, 1H, CH=N), 12.73 (s, 1H, Ar-OH). *Mass (NCI)* m/e: 317 M^+ *For $C_{20}H_{15}NO_3$. R_f=0.79 (1:2,* V/V, *Hexane: Ethyl acetate).*

*(E)-3-Furan-2-yl-1-{4-[(2-nitro-benzylidene)-amino]-phenyl}-propenone (**7**)*

Yellow powder, yield 70%, m.p. 94-96 °C; *IR* (cm^{-1}): 3032 (*C-H Ar*), 2926 (*C-H* aliphatic), 1656 (*C=O*), 1626 (*C=N*), 1593 (*C=C*). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 6.59-6.72 (m, 2H, *CH=CH chalcone, C-H furan), 7.13 (d, j= 3.3 Hz, 1H, C-H furan), 7.15 (d, j= 3.3 Hz, 1H, C-H furan), 7.40 (d, j = 8.5 Hz, 2H, C-H Ar), 7.55 (d, j = 2.3 Hz, 1H, CH=CH chalcone),* 7.60-7.66 (m, 1H, C-*H* Ar*),* 7.79-7.87 (m, 1H, C-*H* Ar*), 7.92 (d, j = 8.5 Hz, 2H, C-H Ar),* 8.11-8.22 (m, 2H, C-*H* Ar*),* 8.93 (s, *1H, CH=N*). *Mass (NCI)* m/e: 346 M^+ *For $C_{20}H_{14}N_2O_4$. R_f=0.70 (1:2,* V/V, *Hexane:Ethyl acetate).*

3.2. Evaluation of antibacterial activities

3.2.1. Determining of antibacterial activity

A well diffusion method was applied to test the antibacterial activity of the synthesized compounds [25]. Pathogenic bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii (Gram-negative), and Staphylococcus aureus* (Gram-positive)) were obtained from the Department of Biology, College of Science, Mustansiriyah University. The plates were prepared by spreading approximately 10^5 CFU/mL of the culture broth of each indicator bacterial isolate on the nutrient agar surface. The agar plates were left for approximately fifteen minutes before aseptically dispensing 50 µL of the tested compounds into the agar wells already bored in the agar plates. The inhibition diameter was measured in millimeter after the plates incubated at 37 °C for 24 hours (Table 1).

3.2.2. Estimation of the minimum inhibitory concentration (MIC) for the synthesized derivatives

The antibacterial evaluation of chalcones containing imine moieties was evaluated by employing a microdilution method using Mueller-Hinton broth. Target compounds were prepared in concentrations ranging from 0.001-0.01 mg/mL, and then 1:2 serial dilutions were achieved by adding broth culture to each compound. Overnight cultures of bacterial species suspension were diluted with physiological sterile solution to 10^8 CFU/mL (turbidity = McFarland barium sulphate standard 0.5). The diluted culture (100 µL) was distributed in multiple plates (96-well plates),

$$\% \text{ Biofilm inhibition} = ((\text{O. D control} - \text{O. D treatment})/\text{O. D control}) \times 100 \quad (1)$$

followed by a sterility control and a growth control (containing culture broth without antibacterial substance). The growth control well and each test were inoculated with a bacterial suspension (5 µL). All experiments were performed in triplicate. All trays were micro-diluted and incubated at 37 °C for 24 hours. After incubation, MICs were measured using an automated or a manual viewing instrument to inspect the growth of each panel well [26].

3.3. Anti-biofilm activity of synthesized derivatives

The anti-biofilm activity of the potent derivatives against bacterial isolates was quantified by co-incubation experiments according to the procedure described in a reported reference [27]. The suspensions of bacterial species were grown for 24 hours in Luria-Bertani broth with glucose (LBG) (0.25 %) at 37 °C. After the incubation period, the medium was diluted (1:50) in freshly prepared LBG pre-warmed to 37 °C. The specific suspension (100 µL) was then added to flat-bottomed microtiter plates (96-well) simultaneously with the target derivatives (100 µL) for each isolate. The positive control wells used in this experiment contained 200 µL of bacterial species suspensions. The plate was covered and locked during incubation at 37 °C for 24 hours. After washing with phosphate-buffered saline (three times), any remaining biofilm was stained with crystal violet 1 % (w/v; 200 µL) for 30 minutes. The plates were dried at room temperature for 15 minutes and washed with PBS again. The dye bound to the adherent plates was solubilized with ethanol/acetone (200 µL) (80:20, v/v) after drying at room temperature, and then an ELISA reader was used to quantify the optical density at 570 nm (OD570). Each measurement was repeated three times. The biofilm inhibition percentages of the tested compounds for each bacterial species were measured as described by the following equation [28]:

3.4. Docking Study

AutoDock version 4.2 package software was used to study the binding of the most effective derivatives (1, 5, 7, and 8) inside the binding pocket of GlcN-6-P synthase. The three

dimensional of enzyme (PDP format) was obtained from Protein Data Bank (PDB code 1MOQ). The enzyme ligand was removed and all the water molecules were extracted. The protein structure was repaired by adding hydrogen atoms to enzyme residues. ChemDraw ultra 7.0 was used to draw the tested compounds. The mol formatted files of the selected derivatives (ligands) were constructed using open Babel 2.3.1 software from the two dimensional structures. The docking study achieved with 62 angstrom in X, Y and Z dimensional with 30.5, 17.5, and -2.2 grids, respectively. The grid points was built on the center of catalytic site and separated by 0.358 angstrom. One hundred runs and one hundred fifty population size was specified as docking simulation using Lamarckian Genetic algorithm. The maximum no.of energy was 25×104 with 2.7×104 generation were utilized [29].

4. Conclusion

New derivatives of Schiff bases (**1-7**) were synthesized and characterized using spectral techniques. The synthesized compounds (**1, 4-7**) exhibited moderate to excellent activity against several antibacterial species. The activity against the biofilm formation of the tested derivatives showed that compound **4** is a lead chemical scaffold for the development of novel anti-biofilm agents. The hit compound (**4**) shows inhibition percentages equal to 66 % and 48 % against *Staphylococcus aureus* and *Acinetobacter baumannii*, respectively. Docking approach was achieved using Auto dock 4.1 to study the binding affinity of the potent discovered hits against the glucoseamine-6-phosphate synthase, the target enzyme for antibacterial agents. Docking study showed that the discovered hits bind the active site in similar manner to enzyme substrate.

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