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Spectroscopic and Biophysical Studies on Chalcones and Schiff Bases Derived from Chromen-2-one and Quinoline-2(1*H*)-one Derivatives as Antibacterial Agents

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A series of chalcone derivatives and arylidene analogues derived from 3-acetyl coumarin were synthesized. The synthesized compounds were elucidated by spectroscopic analysis such as elemental analysis, infrared, ^{1}H & ^{13}C NMR and mass spectroscopies, and then the synthesized compounds were purified and tested against three bacterial strains. Compound **9c** showed high activity against *E. coli* and *P. aeruginosa*. Compounds **4** and **6a** showed moderate activity against *E. coli* while compounds **6a**, **6b** and **9c** showed moderate activity against *S. aureus*. The reference antibiotics were tested against the same bacteria strains in the same conditions and showed that ciprofloxacin have positive activity against *P. aeruginosa* and *S. aureus* but it shows negative activity against *E. coli* while amoxicillin have positive activity against *S. aureus* and negative activity against *E. coli* and *P. aeruginosa*. On the other hand, vancomycin has positive activity against *P. aeruginosa* but not tested against *E. coli* and *S. aureus*. Staph strains were treated with compounds **4** and **7** on DNA fragmentation and DNA cleavage. Docking studies of synthesized compound **9c** was determined and the results were compared with ampicillin. Finally, UV and fluorescence analyses of the synthesized compounds (**3**, **4**, **6b**, **6c**, **6e**, **7**, **9c** and **9e**) were also conducted.

Keywords: 3-Acetyl coumarin, Chalcones, Arylidene derivatives, Molecular docking, Antibacterial activity.

INTRODUCTION

Pechmann condensation method is generally used to synthesized coumarin and their derivatives and exhibits anti-cancer, antifungal and antimicrobial activities [1]. Benzoyl substituted aryl amine, 1,2,3-triazole and coumarin moieties were synthesized and among several substituted compounds, compound *N*-(3,4-dimethoxyphenyl)-4-(4-(2-oxo-2*H*-chromen-4-yl)oxy)-methyl)-1*H*-1,2,3-triazol-1-yl)benzamide showed a potential to inhibit carbonic anhydrase [2]. 4-Hydroxycoumarin was reacted in dry acetone with 3-bromoprop-1-yne and in the presence of K_2CO_3 afforded O-propargylated coumarin, on the other hand, a nucleophilic substitution of propyne amine with 4-bromo-coumarin afforded *N*-propargylated coumarin. These compounds exhibited antimicrobial activities against *Klebsiella*, *Enterococcus*, *Staphylococcus aureus*, *pneumonia, Escherichia coli* and *Pseudomonas aeruginosa* [3,4].

The Schiff bases and chalcones containing pyrimidine were synthesized, chemically elucidated by different spectroscopic analysis [5]. A reactive α,α-unsaturated carbonyl substituted group in chalcone derivatives, demonstrate a wide effect of biological activities such as anti-inflammatory, anti-ulcerative, antiviral, antifungal and antiplatelet [6,7]. The various synthetic method of benzoxazine derivatives including ring expansion [8], intramolecular rearrangement [9], isocyanate precursors [10], *N*-acylantharanilc acid [11], antharanilic acid precursor [12] from chalcones precursors [13,14]. Quinolines and their derivatives can be synthesize by different methods [15] and their Schiff bases synthesized from 2-chloroquinoline-3-carboxaldehyde give moderate inhibition against *Escherichia coli* [16]. In this work, chalcones and Schiff bases derived from coumarin moiety were synthesized, characterized and screened for their preliminary antibacterial activity against selected bacterial stains. Moreover, the molecular docking studies of some target compounds were also carried out and it has been found that some synthesized compounds can bind to Ct-DNA *via* an intercalative mode.

EXPERIMENTAL

Kofler block instruments were used for calculation melting points of the synthesized compounds. Infrared spectra were measured by IC_{50} model FTIR (Thermo) using KBr discs. The

NMR spectra were measured on NMR Spectrometer at 400 MHz for ^{1}H & ^{13}C NMR using TMS as a reference solvent. A thin layer chromatography was carried out using plates Sigma-Aldrich 60 F_{245} (200 μ thickness) to monitor the progress of the reactions.

3-Acetyl coumarin (3): A mixture of salisaldehyde (**1**) (10 mmol) and ethylacetoacetate (10 mmol) was heated under reflux and then few drops of pyridine was added to the reaction mixture. The reaction mixture was refluxed for 1 h (TLC). Then, the result yellow precipitate was filtered off and recrystallized from ethanol to afford yellow powder [17] in 95 % yield, yellow powder, m.p.: 123-125 ºC; R_f: 0.50 (3 % methanol:methylene chloride). ^{1}H NMR (DMSO-d_6): δ ppm: 1.98 (3H, s, $COCH_3$), 7.33-7.91 (4H, m, Ar-H), 8.55 (1H, s, CH); MS *m/z* (%) 188 (M^+).

3-Acetyl-1-aminoquinolin-2(1*H*)-one (4): A mixture of 3-acetyl coumarin (**3**) (0.01 mol) and hydrazine hydrate (0.05 mol) was refluxed for 7 h (TLC). The residue of hydrazine hydrate was evaporated under reduced pressure and the residue was dried and recrystallized from ethanol to give the corresponding 3-acetyl-1-aminoquinolin-2(1*H*)-one (**4**) (**Scheme-I**) in 80% yield, yellow powder, m.p.: 172-174 ºC; R_f: 0.45 (3% methanol: methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3442 (NH_2), 3050 (Ar-H), 1705 (CO), 1720 ($COCH_3$); ^{1}H NMR (DMSO-d_6): δ ppm: 2.10 (3H, s, $COCH_3$), 2.65 (2H, brs, NH_2), 6.80-7.29 (4H, m, Ar-H), 8.25 (1H, s, CH); MS *m/z* (%) 203 (M^++H). Anal. calcd. (found) (%) for $C_{11}H_8O_3$: C, 65.34 (65.25); H, 4.98 (5.01); N, 13.85 (13.92).

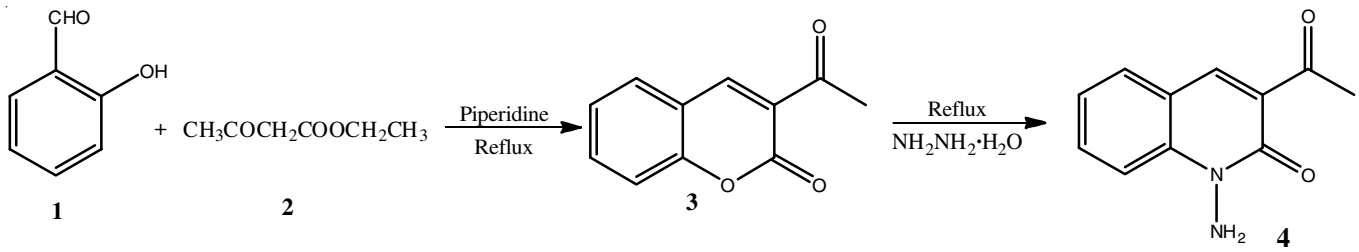
Synthesis of chalcones (6a-e): A mixture of 3-acetyl coumarin (**3**) (0.01 mol) and different aldehydes (**5a-e**) (0.01 mol) in absolute ethanol and in the presence of few drops of piperidine was refluxed for 6 h (TLC). The reaction mixture was cooled and the resulting precipitate was filtered, dried and recrystallized from methanol to give the corresponding chalcone derivatives (**6a-e**) in 85-91% yields (**Scheme-II**).

3-(3-(4-Chlorophenyl)acryloyl)-2*H*-chromen-2-one (6a): Brown powder in 85% yield, m.p.: 202-204 ºC; R_f: 0.72 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3050 (Ar-H), 2940 (CH aliphatic), 1705 (CO), 1720 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.10 (1H, d, *J* = 5.4 Hz, H-10), 7.75 (1H, d, *J* = 5.4 Hz, H-11) 7.40-7.85 (8H, m, Ar-H), 8.55 (1H, s, CH); MS *m/z* (%) 310 (M^++H).

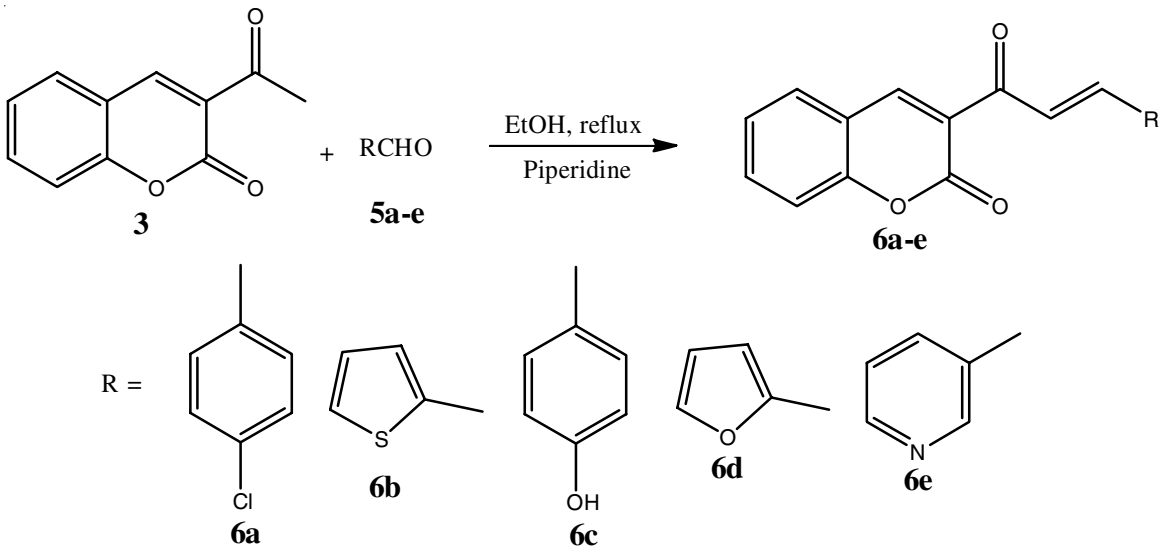
3-(3-(Thiophine-2-yl)acryloyl)-2*H*-chromen-2-one (6b): Red powder in 90% yield, m.p.: 185-187 ºC; R_f: 0.70 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3030 (Ar-H), 2945 (CH aliphatic), 1710 (CO), 1725 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.12 (1H, d, *J* = 5.4 Hz, H-10), 7.67 (1H, d, *J* = 5.4 Hz, H-11) 7.41-7.83 (7H, m, Ar-H), 8.49 (1H, s, CH); MS *m/z* (%) 293 (M^++Na).

3-(3-(4-Hydroxyphenyl) acryloyl)-2*H*-chromen-2-one (6c): Red powder in 88% yield, m.p.: >300 ºC; R_f: 0.45 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3350 (OH), 3090 (Ar-H), 2900 (CH aliphatic), 1700 (CO), 1715 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 5.40 (1H, brs, OH), 7.02 (1H, d, *J* = 5.4 Hz, H-10), 7.87 (1H, d, *J* = 5.4 Hz, H-11) 7.38-7.80 (8H, m, Ar-H), 8.50 (1H, s, CH); MS *m/z* (%) 292 (M^+).

3-(3-(Furan-2-yl) acryloyl)-2*H*-chromen-2-one (6d): Red powder in 91% yield, m.p.: 225-227 ºC; R_f: 0.55 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3080 (Ar-H),



Scheme-I



Scheme-II

2920 (CH aliphatic), 1703 (CO), 1710 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.05 (1H, d, *J* = 5.4 Hz, H-10), 7.66 (1H, d, *J* = 5.4 Hz, H-11) 7.45-7.82 (7H, m, Ar-H), 8.53 (1H, s, CH); MS *m/z* (%) 266 (M^+).

3-(3-(Pyridin-3-yl)acryloyl)-2*H*-chromen-2-one (6e): Yellow crystals in 86% yield, m.p.: 291-293 ºC; R_f: 0.72 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3085 (Ar-H), 2920 (CH aliphatic), 1705 (CO), 1712 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.03 (1H, d, *J* = 5.4 Hz, H-10), 7.81 (1H, d, *J* = 5.4 Hz, H-11), 7.43-7.99 (8H, m, Ar-H), 8.52 (1H, s, CH); MS *m/z* (%) 277 (M^+). Anal. calcd. (found) % for $C_{17}H_{11}NO_3$: C, 73.64 (73.55); H, 4.00 (4.05); N, 5.05 (4.97).

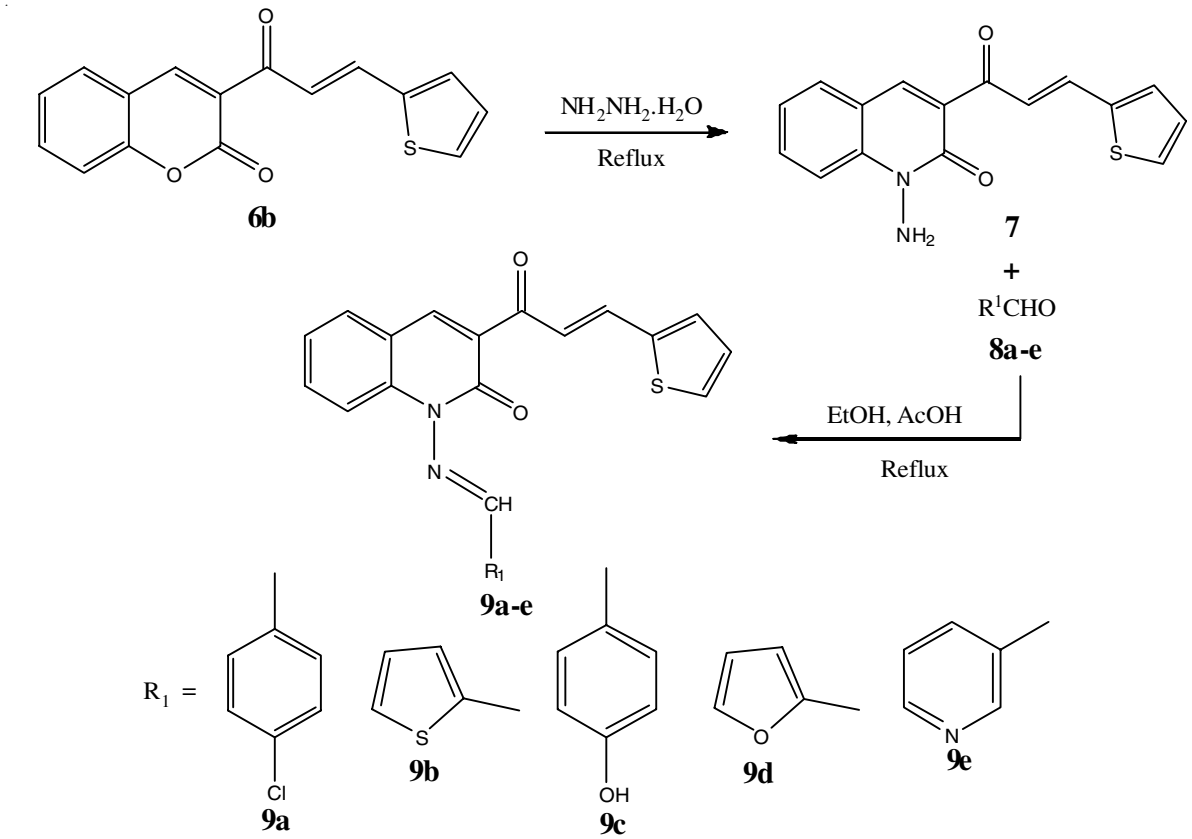
Synthesis of 1-amino-3-(3-(thiophene-2-yl) acryloyl) quinoline-2(1*H*)-one (7): A mixture of 3-(3-(thiophine-2-yl)-acryloyl)-2*H*-chromen-2-one (**6b**) (0.01 mol) and hydrazine hydrate (0.05 mol) was refluxed for 10 h (TLC). The residue of hydrazine hydrate was evaporated under reduced pressure and the residue was dried and recrystallized from ethanol to give the corresponding 1-amino-3(3-(thiophene-2-yl)acryloyl)-quinolin-2(1*H*)-one (**7**) in 73% yield, white powder, m.p.: 234-236 ºC; R_f: 0.65 (3% methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3370 (NH_2), 3060 (Ar-H), 2895 (CH aliphatic), 1700 (CO), 1715 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 2.45 (2H, brs, NH_2), 6.78-8.13 (7H, m, Ar-H), 7.06 (1H, d, *J* = 5.4 Hz, H-10), 7.65 (1H, d, *J* = 5.4 Hz, H-11) 8.32 (1H, s, CH); MS *m/z* (%) 298 (M^++2H). Anal. calcd. (found) % for $C_{11}H_8O_3$: C, 64.85 (64.80); H, 4.08 (4.11); N, 9.45 (9.54).

Synthesis of Schiff bases (9a-e): A mixture of compound **7** (0.01 mol) and appropriate aldehyde derivatives (**8a-e**) in absolute ethanol and in the presence of acetic acid as catalyst was heated under reflux for 4 h (TLC), then the mixture was cooled and the resulting precipitate was filtered, dried and recrystallized from ethanol to afford the corresponding Schiff bases **9a-e** in 77-88% yields (**Scheme-III**).

1-(4-Chlorobenzylidine)amino-3(3-(thiophene-2-yl)acryloyl)quinoline-2(1*H*)-one (9a): Pale yellow powder in 83% yield, m.p.: 250-252 ºC; R_f: 0.45 (3% methanol: methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3090 (Ar-H), 2950 (CH aliphatic), 1700 (CO), 1707 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.05 (1H, d, *J* = 5.4 Hz, H-10), 7.70 (1H, d, *J* = 5.4 Hz, H-11), 7.33-8.14 (11H, m, Ar-H), 8.38 (1H, s, CH), 8.52 (1H, s, CH); MS *m/z* (%) 418 (M^+). Anal. calcd. (found) (%) for $C_{23}H_{15}N_2O_2SCl$: C, 65.95 (66.02); H, 3.61 (3.75); N, 6.69 (6.48).

3-(3-(Thiophen-2-yl)acryloyl)-1-(thiophene-2-yl)-methylene)amino)quinoline-2(1*H*)-one (9b): Pale yellow powder in 80% yield, m.p.: 211-213 ºC; R_f: 0.45 (3 % methanol: methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3070 (Ar-H), 2910 (CH aliphatic), 1705 (CO), 1713 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.07 (1H, d, *J* = 5.4 Hz, H-10), 7.63 (1H, d, *J* = 5.4 Hz, H-11), 7.35-8.10 (10H, m, Ar-H), 8.35 (1H, s, CH), 9.23 (1H, s, CH); MS *m/z* (%) 390 (M^+). Anal. calcd. (found) (%) for $C_{21}H_{14}N_2O_2S_2$: C, 64.59 (66.02); H, 3.61 (3.75); N, 7.17 (6.48).

1-(4-(Hydroxybenzylidine)amino)-3-(thiophene-2-yl)acryloyl)quinoline-2(1*H*)-one (9c): Pale yellow powder in 77 % yield, m.p.: > 300 ºC; R_f: 0.72 (3 % methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3075 (Ar-H), 2900 (CH aliphatic), 1705 (CO), 1710 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 5.30 (1H, brs, OH), 7.00 (1H, d, *J* = 5.4 Hz, H-10), 7.60



Scheme-III

(1H, d, *J* = 5.4 Hz, H-11), 6.80-8.11 (11H, m, Ar-H), 8.39 (1H, s, CH), 8.54 (1H, s, CH); MS *m/z* (%) 400 (M^+). Anal. calcd. (found) % for $C_{23}H_{16}N_2O_3S$: C, 68.98 (69.00); H, 4.03 (3.95); N, 7.00 (6.88).

1-(Furan-2-methylene)amino)-3-(3-(thiophene-2-yl)acryloyl)quinolone-2(1*H*)-one (9d): Brown powder in 88 % yield, m.p.: 276-278 ºC; R_f: 0.45 (3 % methanol:methylene chloride). IR spectra (KBr, ν_{max}, cm^{-1}): 3075 (Ar-H), 2880 (CH aliphatic), 1700 (CO), 1710 (CO); ^{1}H NMR (DMSO-d_6): δ ppm: 7.01 (1H, d, *J* = 5.4 Hz, H-10), 7.62 (1H, d, *J* = 5.4 Hz, H-11), 6.50-8.16 (10H, m, Ar-H), 8.11 (1H, s, CH), 8.37 (1H, s, CH); MS *m/z* (%) 375 (M^++H). Anal. calcd. (found) % for $C_{21}H_{14}N_2O_3S$: C, 67.37 (67.49); H, 3.77 (3.85); N, 7.48 (7.21).

1-(Pyridin-3-methylene)amino)-3(3-(thiophene-2-yl)acryloyl)quinolone-2(1*H*)-one (9e): Brown powder in 85 % yield, m.p.: 296-298 ºC; R_f: 0.55 (3 % methanol:methylene chloride). IR (KBr, ν_{max}, cm^{-1}): 3070 (Ar-H), 2870 (CH aliphatic), 1695 (CO), 1705 (CO); ^{1}H NMR (DMSO-d_6): δ (ppm): 7.00 (1H, d, *J* = 5.4 Hz, H-10), 7.68 (1H, d, *J* = 5.4 Hz, H-11), 7.37-9.00 (11H, m, Ar-H), 8.22 (1H, s, CH), 8.42 (1H, s, CH); MS *m/z* (%) 385 (M^+). Anal. calcd. (found) for $C_{22}H_{15}N_3O_2S$: C, 68.55 (68.63); H, 3.92 (3.77); N, 10.90 (10.83).

Bacterial isolates: Microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *pseudomonas aeruginosa*) used in this study were obtained from King Faisal Hospital after receiving the ethical approval from Taif Directorate of Health Affairs, Taif, Saudi Arabia.

Antimicrobial assay: The antibacterial activity of five synthesized compounds (**9c**, **6a**, **6b**, **4** and **7**) were carried out using agar well diffusion test with minor modifications [18]. Agar poured into sterile petri-dishes and allowed to harden, then 1 mL of each tested bacterial isolates inoculum equal to 0.5 McFarland standard was inoculated into the Mueller agar plates. A 30 µL of stock concentration (100 mg/mL) diluted in DMSO from each synthesized compounds was added into the agar wells. The plates were incubated at 37 ºC for 24 h. Positive and negative control discs for each bacterial isolate including ciprofloxacin (5 µg/mL) and amoxicillin (10 µg/mL) for *Escherichia coli* and *Pseudomonas auroginosa*; and amoxicillin (10 µg/mL) and vancomycin (10 µg/mL) for *S. aureus* were prepared.

Determination of minimum inhibitory concentration (MIC): The MIC values were determined using broth microdiltion test in 96 well microtiter plate with triplicates and with minor modifications [19]. A 100 µL of DMSO was added to the wells and 100 µL of stock concentration (100 mg/mL DMSO) of each synthesized compound was added and thus double fold serial dilution was achieved followed by a addition of 100 µL of sterile double strength Mueller-Hinton broth was to each well. Enrichment of all bacterial isolates were done on Mueller Hinton broth and incubated over night at 37 ºC with agitation. A 100 µL of 0.5 McFarland standards of each inoculum was added to all tested wells incubated and finally the plates were again incubated at 37 ºC for 24 h. Turbidity was measured at 600 nm using microtiter plate reader. A negative control (blank) was taken as Mueller Hinton broth without any agents and positive control as medium with agents.

Treatment of staph strains with compounds 4 and 7 on DNA fragmentation and DNA cleavage: A purified colonies of *S. aureus* strains were grown in trypticase soya broth for 24 h at 37 ºC. For DNA cleavage assay, bacterial colonies were incubated with either compounds 4 and 7 at a dose of 100 mg/mL DMSO for 4, 8, 24, 48 and 72 h. Bacterial broth was precipitated after centrifugation at 10000 rpm for 10 min. The pellets of colonies (200 colonies) were suspended in 500 µL DEPC water and heated at 100 ºC in vortex well for 2 min. Supernatant clear fluid was taken and a double volume of ice cold absolute ethanol were added and incubated for overnight at -20 ºC. Contents were centrifuged at 12000 rpm for 15 min and then discard supernatant. A DNA pellet were washed with 70 % ethanol, dried in air and then dissolved in double distilled water. A DNA concentration was measured in BIORAD spectrophotometer at O.D. 260 nm. Then 250 ng of extracted and purified DNA was loaded in 1.2 agarose gel stained with ethidium bromide and photocopied using gel documentation system (Bio-Rad, Co., USA).

Computational study: Target compounds were built and minimized their energy with PM3 through MOPAC then DFT using B3LYP/6-311G. All the quantum chemical computations were performed using the PM3 semi-empirical Hamiltonian molecular orbital calculation MOPAC16 package [20], then employed DFT in Gaussian 09 W program package [21] with Becke3-Lee-Yang-parr (B3LYP) level using 6-311G* basis as implemented in MOE 2015 package [22].

Selection of proteins structure: Docking experiment was carried out for the target active site into DNA Gyrase B (ID: 2EX6) using MOE 2015 . The errors of active sites were corrected by the structure preparation process in MOE. After correction, hydrogens were added and then partial charges were calculated. Energy minimization (AMBER12:EHT, root mean square gradient: 0.100) was also performed.

Binding site analysis: The binding site of each receptors were identified through the MOE Site Finder program, which uses a geometric approach to calculate putative binding sites in a protein, starting from its tridimensional structure. This method is based on alpha spheres, which are generalization of convex hulls. The prediction of binding sites, performed by the MOE Site Finder module, confirmed the binding sites defined by co-crystallized ligands in the holo-forms of the investigated proteins.

RESULTS AND DISCUSSION

In this work, salicylaldehyde (**1**) was reacted with ethylacetoacetate (**2**) in the presence of pyridine as base under reflux to give 3-acetyl coumarin (**3**) in 95% yield. Structural analyses were done with ^{1}H NMR showed that a singlet at 1.98 ppm for $COCH_3$ and multiplet at 7.33-7.91 ppm for CH aromatic. The mass analysis showed that *m/z* % 188 (M+). 3-Acetyl coumarin (**3**) was reacted with hydrazine hydrate under reflux to give 3-acetyl-1-aminoquinolin-2(1*H*)-one (**4**) in 80% yield. Structural analyses were done with FT-IR, showed that peak at 3442 cm^{-1} for NH_2, peak at 1720 cm^{-1} for $COCH_3$ and done with ^{1}H NMR showed that a singlet at 2.10 ppm for $COCH_3$, abroad at 2.65 ppm for NH_2, a multiplet at 6.80-7.29 ppm for aromatic system

and a singlet at 8.25 for CH. The mass spectra showed that *m/z* % 203 (M^++H) (**Scheme-I**).

3-Acetyl coumarin (**3**) was reacted with appropriate aldehydes (**5a-d**) in the presence of pipridin as base under reflux to give the corresponding chalcones **6a-d** in 85-91 % yields, respectively. Structural analyses were done with ^{1}H NMR for compound **6a** showed that a doublet at 7.10 ppm for CH (H-10), a doublet at 7.75 ppm for CH (H-11), a multiplet at 7.40-7.85 for CH aromatic and a singlet at 8.55 for CH. The mass spectra showed that *m/z* % 310 (M^++H). ^{1}H NMR for compound **6b** showed that a doublet at 7.12 ppm for CH (H-10), a doublet at 7.67 for CH (H-11), a multiplet at 7.41-7.83 ppm for CH aromatic and a singlet at 8.49 for CH. The mass spectra showed that *m/z* (%) 293 (M^++Na). ^{1}H NMR for compound **6c** showed that a broad at 5.40 for OH, a doublet at 7.02 ppm for CH (H-10), a doublet at 7.87 ppm for CH (H-11), a multiplet at 7.38-7.80 ppm for CH aromatic and a singlet at 8.50 ppm for CH. The mass spectra showed that *m/z* % 292 (M^+). ^{1}H NMR for compound **6d** showed that a doublet at 7.05 ppm for CH (H-10), a doublet at 7.66 ppm for CH (H-11), a multiplet at 7.45-7.82 ppm for CH aromatic and a singlet at 8.53 for CH. The mass spectra showed that *m/z* % 266 (M). ^{1}H NMR for compound **6e** showed that a doublet at 7.03 ppm for CH (H-10), a doublet at 7.81 ppm for CH (H-11), a multiplet at 7.43-7.99 ppm for CH aromatic and a singlet at 8.52 ppm for CH. The mass spectra showed that *m/z* (%) 277 (M^+) (**Scheme-II**).

3-(3-(Thiophine-2-yl)acryloyl)-2*H*-chromen-2-one (**6b**) was reacted with appropriate aldehydes hydrazine hydrate under reflux to give hydrazide **7** in 73% yield. Structural analyses were done with ^{1}H NMR for compound **7** showed that a broad at 2.45 ppm for NH_2, a multiplet at 6.78-8.13 ppm for CH aromatic, a doublet at 7.06 ppm for CH (H-10), a doublet at 7.65 ppm for CH (H-11) and a singlet at 8.32 ppm for (CH). The mass spectra showed that *m/z* % 298 (M^++2H). 1-Amino-3-(3-(thiophene-2-yl)acryloyl)quinolin-2(1*H*)-one (**7**) was reacted with appropriate aldehydes **8a-d** in absolute ethanol and in the presence of acetic acid as catalyst under reflux to give the corresponding Schiff bases **9a-d** in 77-88% yields, respectively. Structural analyses were done with ^{1}H NMR for compound **9a** showed that a doublet at 7.05 ppm for CH (H-10), a doublet at 7.70 ppm for CH (H-11), multiplet at 7.33-8.14 ppm for CH aromatic, 8.38 ppm for CH and a singlet at 8.52 ppm for CH. The mass spectra showed that *m/z* % 418 (M+). ^{1}H NMR for compound **9b** showed that a doublet at 7.07 ppm for CH (H-10), a doublet at 7.63 ppm for CH (H-11), multiplet at 7.35-8.10 ppm for CH aromatic, 8.35 ppm for CH and a singlet at 9.23 ppm for CH. The mass spectra showed that *m/z* % 390 (M+). Similarly, compound **9c** showed that a broad at 5.30 ppm for OH, a doublet at 7.00 ppm for CH (H-10), a doublet at 7.60 ppm for CH (H-11), multiplet at 6.80-8.11 ppm for CH aromatic, 8.39 ppm for CH and a singlet at 8.54 ppm for CH. The mass spectra showed that *m/z* % 400 (M+). ^{1}H NMR for compound **9d** showed that a doublet at 7.01 ppm for CH (H-10), a doublet at 7.62 ppm for CH (H-11), multiplet at 6.50-8.16 ppm for CH aromatic, 8.11 ppm for CH and a singlet at 8.37 ppm for CH. The mass spectra showed that *m/z* % 375 (M^++H). ^{1}H NMR for compound **9e** showed that a doublet at 7.00 ppm for CH (H-10), a doublet at 7.68 ppm for CH (H-11), multiplet at 7.37-9.00 ppm for CH aromatic, 8.22 ppm for CH and a singlet at 8.42 ppm for CH. The mass spectra showed that *m/z* % 385 (M+).

Antibacterial activity: The antibiogram activity of five synthesized compounds (**4**, **7**, **6a**, **6b** and **9c**) were tested against three bacterial strains using agar well diffusion test. Table-1 shows that compound **9c** (100 mg/mL DMSO) was the most prominent suppressive substance against *E. coli* followed by *P. aeruginosa* with inhibition zone (30 and 25mm), respectively. All the tested *E. coli* isolates were sensitive to compounds **4** and **6a** with zone size 15 and 13 mm. *S. aureus* was sensitive to compound **6a**, **6b** and **9c** with inhibition zone measured 12, 12 and 16 mm, respectively meanwhile, compound **7** showed the highest antibacterial effect against *S. aureus* with zone size equal 18 mm.

TABLE-1
ANTIMICROBIAL ACTIVITY OF TESTED CHEMICAL SUBSTANCES AND ANTIBIOTICS OF THREE CLINICAL ISOLATES

Substance code	Inhibition zone (mm) of 100 mg/mL		
	E. coli	*P. aeruginosa*	*S. aureus*
9c	30	25	16
4	15	No zone	12
6a	13	No zone	12
7	No zone	No zone	18
6b	No zone	No zone	11
Ciprofloxacin	No zone	< 6	22
Amoxicillin	No zone	No zone	< 6
Vancomycin	Not tested	Not tested	19

All chemical substances showed antibacterial activity on agar well diffusion test were examined by the MIC test for determination of the lowest concentration of each chemical substance that inhibit a visible bacterial growth in microliter plate with 96 well. The obtained results showed variances in MIC values with all tested compounds. Concerning *S. aureus*, compound **6b** showed the lowest MIC and MBC values (0.39/0.78 mg/mL) followed by compounds **4**, **7**, **9c** and **6b** (0.78/1.56, 6.25/12.5, 25/50 and 25/50 mg/mL), respectively. Compounds **4**, **9c** and **6a** were also active against *E. coli* and MIC and MIBC values were (0.195/0.39, 12.5/25 and 6.25/12.5 mg/mL), respectively. Finally, *P. aeruginosa* showed MIC and MBC values only with compounds **4** and **9c** (0.195/0.39 and 12.5/25 mg/mL) as shown in Table-2. The antimicrobial effect of chalcones is often due to the presence of -OH groups in various positions of B ring [23]. Reduction of MICs values, of chalcones making

TABLE-2
MIC TEST OF THREE CLINICAL ISOLATES AGAINST 5 DIFFERENT SYNTHESIZED COMPOUNDS

Substance code	MIC and MBC by mg/mL		
	E. coli	*P. aeruginosa*	*S. aureus*
9c	12.5/25	12.5/25	25/50
4	0.195/0.39	0.78/1.56	0.78/1.56
6a	6.25/12.5	Not tested	25/50
7	Not tested	Not tested	12.5/6.25
6b	Not tested	Not tested	0.39/0.78

them more suitable for therapeutic usage as alternative to the commonly used antibiotics [24].

Treatment of staph strain with complexes 4 and 7 on DNA fragmentation and DNA cleavage: The formation of clear DNA smear in case of treated samples indicate that programmed cell death of *S. aureus* isolate treated with constant concentration at different time intervals with smearing and fragmented DNA [25]. As shown in Fig. 1, an incubation of *Staphylococcus aureus* with compounds **7** (lanes 2-7) and **4** (lanes 8-13) in a concentration of (100 mg/mL DMSO) for 4, 8, 24, 48 and 72 h. Lanes 2 and 8 are untreated bacteria showed a bacterial DNA without clear apparent cleavage and degradation. From 4 h for both complexes DNA were degraded in time dependent manner reaching its final degradation and no any smears can be seen at 72 h. At 72 h, DNA degradation was completely induced with 100 % and did not appear in stained gel compared to untreated bacteria (lane 2 and 8) and 4 h treatments.

Docking studies: In order to evaluate the binding affinity of the most active compound toward the catalytic site of the target enzyme, the docking study was carried out. The best binding affinity, expressed as the highest variation of Gibb′s free energy (ΔG) of the complex with the target penicillin-binding protein (PDB code: 2EX6), which are implicated in

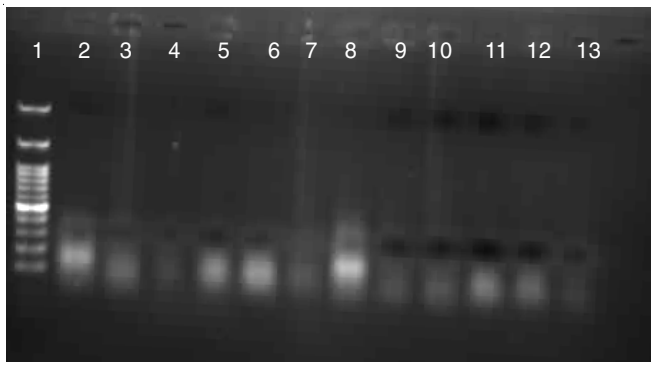


Fig. 1. DNA Cleavage activity of compounds **7** and **4** on *Staphylococcus aureus* growth. Lane 1: DNA ladder. Lane 2: *Staphylococcus aureus* control without any treatment. Lane 3-7 compound **7** at 4, 8, 24, 48 and 72 h. Lane 8 is untreated bacteria, lanes from 9-13 are incubated bacteria with compound **4** for 4, 8, 24, 48 and 72 h, respectively

maturation of bacterial cell wall and formation of cell shape. The crystallographic structure of 2EX6 included ampicillin as the docked ligand at the binding pocket that showed a suitable recognition with the conserved amino acid residues (Fig. 2). The molecular docking study of synthesized compound **9c** showed ΔG= -5.645 Kcal/mol, which exhibited a H-bond with

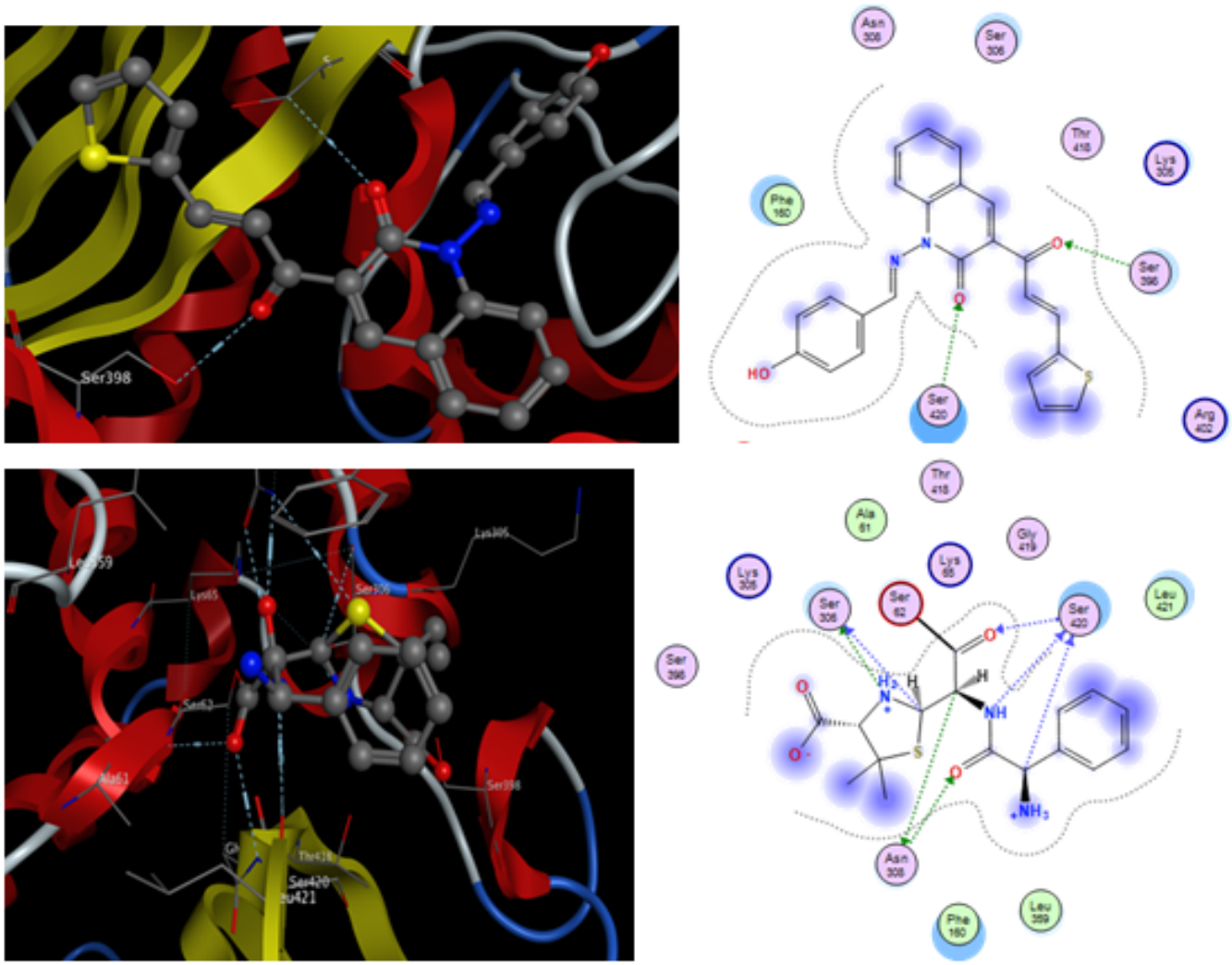


Fig. 2. Binding mode and residues involved in ampicillin and compound **9c** docked and geometrically optimized in the 2EX6 binding pocket

important amino acid residues *viz*. Ser420 and Ser398. While the reference drug ampicillin showed the value of ΔG = -5.506 Kcal/mol (Table-3). The influence of structural flexibility on ΔG is also supported by the dispersion of Gibb's free energy values and the large spatial dispersion of the predicted poses (Table-3).

Structure activity relationship (SAR): The molecular docking studies of the newly synthesized compound **9c** come in agreement with the corresponding experimental antimicrobial results. It was found that the adjacent thiophene core in parent quinoline compound lead that exhibited antibacterial effect greater than other members. This compound stabilized

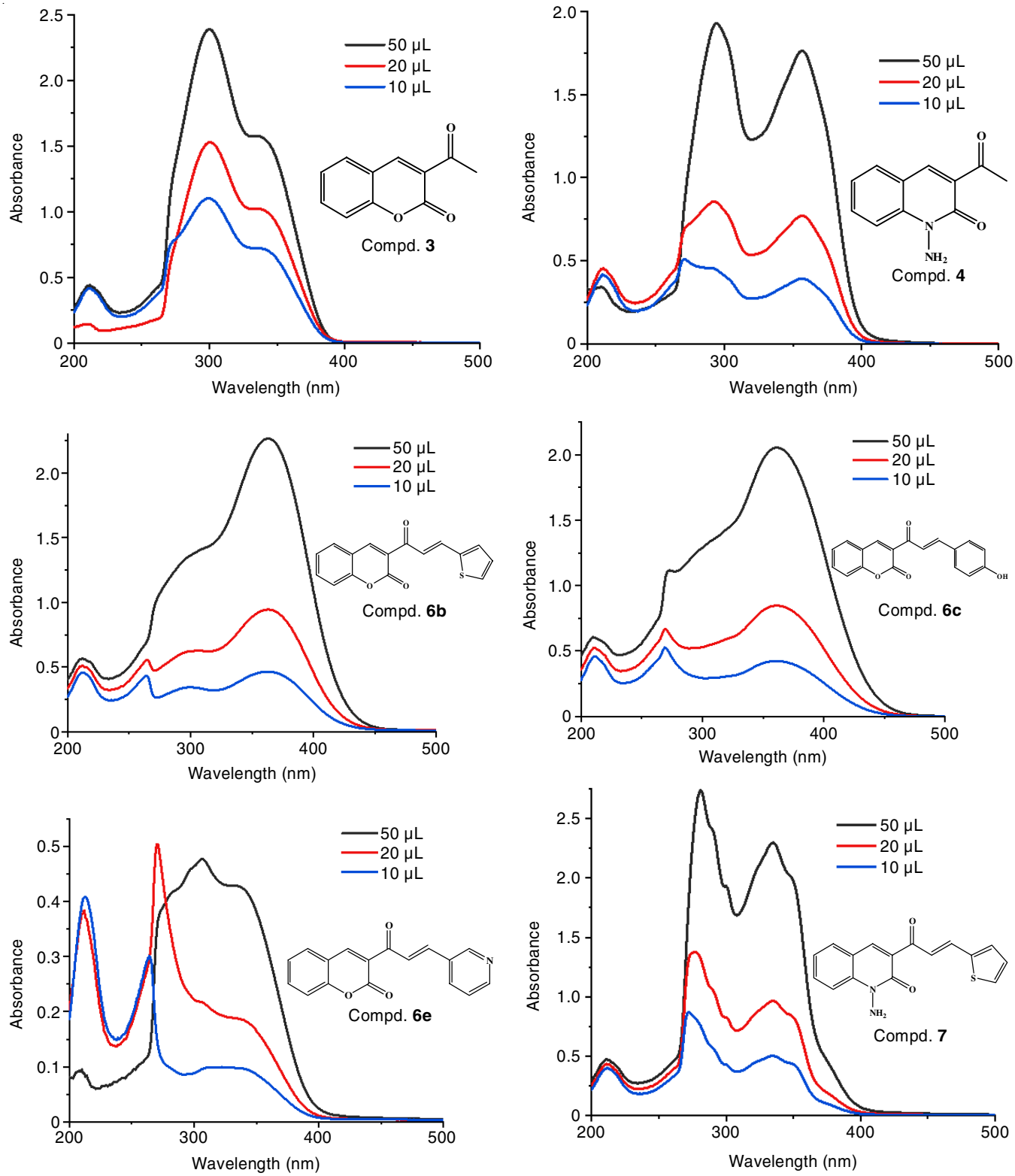


Fig. 3. UV spectra of synthesized compounds (**3**, **4**, **6a**, **6b**, **6c**, **6e** and **7**)

TABLE-3
DOCKING ENERGY SCORES (kcal/mol) WITH KEY INTERACTIONS WITH ACTIVE SITES DERIVED FROM THE MOE FOR **9c** AND AMPICILLIN

Parameters	Ampicillin	**9c**	Parameters	**9c**	
ΔG	-5.560	-5.646	Ligand	O	O
RMSD	1.7	1.7	Receptor	SER-398	SER-420
$E_{Int.}$	-38.52	54.167	Interaction	H-acceptor	H-acceptor
E_{place}	-62.28	-36.15	Distance (Å)	3	3.39
$E_{conf.}$	-13.57	-10.53	E (kcal/mol)	-2.5	-1
$E_{ele.}$	-23.78	-34.01			

ΔG = Free binding energy of the ligand from a given conformer, E_{conf} = Free binding energy of the ligand from a given conformer; E_{place} = Free binding energy of the ligand from a receptor. $E_{Int.}$ = Affinity binding energy of ligand with receptor; E_{ele} = Electrostatic interaction with the receptor. RMSD = The root mean square deviation of the docking pose compared to the co-crystal ligand position.

in binding pocket by perpendicular arranged of quinoline and thiophene with Ser420. The interaction mode of ligand with hydrophilic amino acids backbone in binding site (Fig. 2) postulated that the hydrophobicity is an important pharma biotic character for penetration molecule *via* biological system.

Physical studies: Fig. 3 shows the optical absorption spectra of compounds (**3**, **4**, **6a**, **6b**, **6c**, **6e** and **7**) with increasing concentrations. The concentrations of 50, 20 and 10 µL were determined at 350, 370, 340, 380, 300, 290 nm and 310 nm, respectively and it is noticed that absorption is shifted to longer wavelengths from 290 to 370 nm.

Fig. 4 shows the fluorescence spectra of synthesized compounds (**3**, **4, 6b**, **6c**, **6e**, **7** and **9e**), where compound **3** emitted the exciting light at 480 nm. Similarly, compounds **4**, **6b**, **6c**, **6e**, **7** and **9e** emitted the exciting light at 460, 450, 500, 470, 480 and 470 nm, respectively.

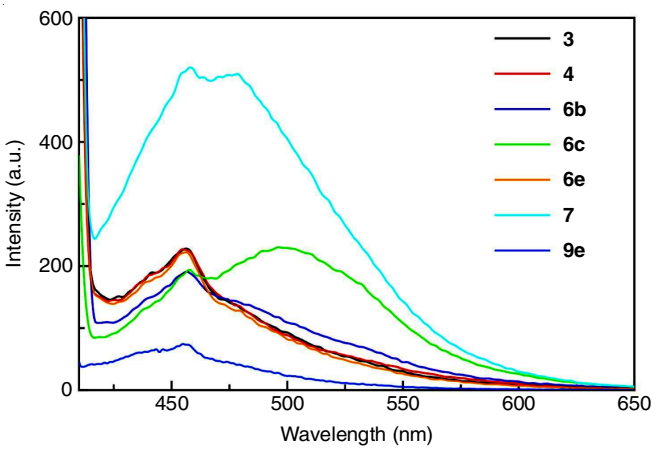


Fig. 4. Fluorescence spectra of synthesized compounds (**3**, **4**, **6b**, **6c**, **6e**, **7** and **9e**)

Conclusion

In this work, chalcones and arylidene derivatives derived from coumarin were synthesized and elucidated with spectroscopic analysis and also the molecular docking studies were conducted in order to identify the theoretical binding with DNA of bacteria. The bacterial activity of five synthesized compounds (**4**,**7**, **6a**, **6b** and **9c**) were tested against three bacterial strains and also tested for the DNA cleavage and DNA fragmentation.

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CONFLICT OF INTEREST

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

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