RESEARCH ARTICLE

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Molecular Docking Studies and Microbial Activities of Mono-, Di- and Tri-Substituted Simple Coumarins

Hülya Çelik Onar, Emel Mataracı Kara, Deniz Ceyhan, and Erol Erçağ

ABSTRACT

In this study, thirteen simple coumarin derivatives were evaluated for antibacterial and antifungal activities. The test results showed that the coumarin derivatives used, especially the 8, 11, 12 and 13 derivatives, were more susceptible to gram positive bacteria. Furthermore, the antifungal activity of compound 11 was observed to be promising. Insertion analyzes were applied to elucidate the interaction mechanisms between coumarin compounds and target proteins (selected from S. aureus and C. albicans). Compound 11 exhibited high binding affinity for CYP51 (-7.32 kcal/mol) and strong protein-ligand molecular interactions. As a result, it is stated that 11 is open to various chemical modifications and has a good initial skeletal molecular structure for antifungal compound designs.

Keywords: Antibacterial Activity, Antifungal Activity, Coumarin, Molecular Docking.

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I. Introduction

The main aim of the researchers is to discover inexpensive and effective new drugs or improve existing ones. In recent years, the gradual increase in deaths due to infections caused by microorganisms and the development of resistance of microorganisms to antifungal and antibacterial agents necessitated the discovery of new antibiotics. The interest that started in the past in coumarin and its derivatives still continues.

Coumarins with benzo-α-pyranolactone ring structure have a wide range of biological activity which as antimicrobial, anti-diabetic, anticoagulant, antitumor, antioxidant, cardiotonic, anti-Alzheimer's, anti-inflammatory and anti-HIV activities [1], [2]. Many studies on antibacterial and antifungal activities in the literature generally include hybride coumarins which are fused with groups such as thiazole [3], [4], imidazole [5], [6], pyridine [7], pyrazole [8], pyrano [9], pyrimidine [10]-[12], quinoxalone [13], and metal complex [14]-[17]. There are also a few studies on the antimicrobial activities of simple coumarins in the literature [18]-[21].

In general, as it can be understood from the literature review, the antibacterial and antifungal effects of complicated coumarins were investigated. In this paper, we studied the antibacterial and antifungal effects of simple coumarins (mono-, di-, and tri-substituted), which are much easier to synthesize. Furthermore, Molecular docking studies were performed to understand the possible binding efficiency and mode of action of the synthesized core compounds and reference model inhibitor molecules with bacterial and fungal key target proteins.

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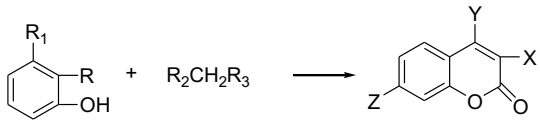
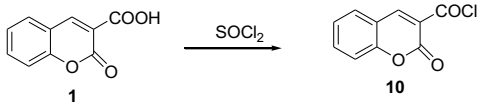
II. EXPERIMENTAL

A. Synthesized Coumarin Derivatives

In our previous publication, mono-, di- and tri-substituted coumarins were synthesized using Pechmann, Knoevenagel and Duff reactions and evaluated for their antioxidant activities [22]. The coumarins to be used in this study are shown in (1)-(3). Table I shows synthesized coumarin derivatives.



(1)



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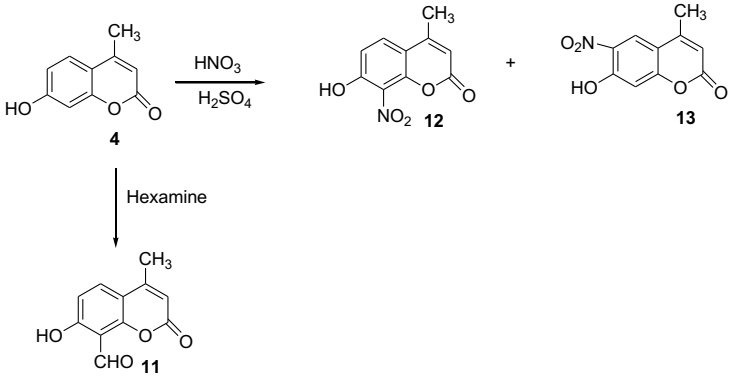


(3)

TABLE I:SYNTHESIZED COUMARIN DERIVATIVES

R	R_1	R_2	R_3	X	Y	Z	Compound
CHO	H	COOH	COOH	COOH	H	H	**1**
CHO	H	$COCH_3$	$COOC_2H_5$	$COCH_3$	H	H	**2**
CHO	H	COC_6H_5	$COOC_2H_5$	COC_6H_5	H	H	**3**
H	OH	$COCH_3$	$COOC_2H_5$	H	CH_3	OH	**4**
H	NH_2	$COCH_3$	$COOC_2H_5$	H	CH_3	NH_2	**5**
H	OCH_3	$COCH_3$	$COOC_2H_5$	H	CH_3	OCH_3	**6**
H	NH_2	COC_6H_5	$COOC_2H_5$	H	C_6H_5	NH_2	**7**
H	OH	COC_3H_7	$COOC_2H_5$	H	C_3H_7	OH	**8**
H	OH	COC_6H_5	$COOC_2H_5$	H	C_6H_5	OH	**9**

B. In Vitro Antimicrobial Activity

In vitro antimicrobial activities of 13 different coumarin molecules against ten different ATCC isolates (four Gram negative, three Gram positive and three fungi) were investigated. MICs of the compounds were identified by the broth microdilution technique as approved by the Clinical and Laboratory Standards Institute [23], [24]. Molecules were dissolving in Dimethyl sulfoxide (DMSO, Sigma), and serial twofold dilutions (2500 to 1.22 µg/mL) were prepared in Mueller Hinton broth (MHB) for bacteria and RPMI-1640 medium for yeast. Bacterial inoculums were prepared with an approximately 5h MHB culture and a different microplate was used for each microorganism. Then, each individual bacterial inoculum was spectrophotometrically arranged with turbidity equivalent to a 0.5 McFarland and additionally diluted in MHB to obtain a final concentration of 5×10^5 CFU/mL in the test tray. Candida albicans ATCC 10231, C. parapsilosis ATCC 22019, and C. tropicalis ATCC 750 were prepared in RPMI-1640 medium to obtain a final concentration of 5×10^3 CFU/mL. Each test tray was stored in a plastic container to evade evaporation. Incubation: It was done for 18-24 hours at 37 °C for trays containing MHB, and for 48 hours at 37 °C for those existing RPMI-1640 medium. MIC was identified as the lowest concentrations of compounds that produced visible inhibition of apparent growth. The standard antimicrobials were also studied against the tested microorganisms.

C. Molecular Docking Studies

Molecular docking studies were performed to understand the possible binding efficiency and mode of action of the synthesized core compounds and reference model inhibitor molecules with bacterial and fungal key target proteins. The Molecular Operating Environment software package (MOE, v2019.0102, Chemical

Computing Group ULC) [25] was used for molecular docking calculations and molecular visualization of docking results. Preparation of synthesized compounds (1-13) and model inhibitor molecules (amikacin, ceftazidine and cefuroxime) for molecular docking were carried out by using MarvinSketch software [26]. These molecular structures have been protonated, were charges added and conformation minimization was performed with the Root Mean Square gradient (RMS 0.01 kcal/mol/A2) by using the MMFF94 Forcefield parameters, which can be accessed in Energy Minimization protocols of these softwares [27].

Docking studies of compounds (1-13) and some reference compounds were carried out for three different important target structures named DNA gyrase and Aminoglycoside Phosphotransferase (2")-Ia from Staphylococcus aureus, sterol 14-alpha demethylase (CYP51) from Candida albicans. In addition, docking calculations of the studied compounds were made against the bacterial ribosomal decoding site, which is the main antibiotic target structure. The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank was used to provide three-dimensional crystal structures of these target proteins [28]. For use in docking calculations, structures with PDB IDs of XCS [29] for DNA gyrase, 6CGD [30] for Aminoglycoside Phosphotransferase (2")-Ia, 5TZ1 [31] for sterol 14-alpha demethylase and 4P20 [32] for bacterial ribosomal decoding site were chosen as crystal structure models corresponding to these target proteins. The structure defects in the PDB protein models determined for the docking calculations were corrected with the "Structure Preparation" module of the MOE software according to the default parameters. Energy minimizations of proteins or protein-ligand complex systems were performed according to the MOE Energy Minimization Protocols [33], [34]. Possible ligand binding sites in protein models were determined with the MOE “SiteFinder” module, which uses a geometric method based on Alpha Shapes, a generalization of convex surfaces developed by Edelsbrunner [35]. Local docking of new synthesized compounds and model inhibitors to the active site of these targets proteins was performed via MOE using the default docking calculation parameters.

III. Results and Discussion

A. In Vitro Antimicrobial Activity

The in vitro antimicrobial activity of 13 coumarin derivatives against three Gram-positive bacteria, four Gram-negative bacteria, and three fungi by the broth microdilutions technique using the CLSI recommendations [23], [24]. The well-known commercial antibiotics were used as the standard drugs and the minimal inhibitory concentrations (MIC) values compared with the standard drugs presented in Table 1 and 2. Depending on the antibacterial results for all compounds, the test-culture P. aeruginosa appeared to be resistant to all synthesized compounds. We also observed that 10 of 13 coumarin derivatives displayed no inhibitory activity against all Gram-negative bacteria. Concerning the antibacterial activity, the Gram-positive bacteria were more susceptible to the coumarin molecules than the Gram-negative ones. Generally, the findings showed that some compounds displayed varying effects on the growth of the tested Gram-positive bacterial strains. The results showed that all coumarin derivatives exhibited antimicrobial activity against E. faecalis except **3** and **9**. In addition to this, **8**, **11**, **12**, and **13** exhibited potent antibacterial potential against S. aureus and S. epidermidis with an MIC range of 78.12 to 156.2 µg/mL values. Specifically, **11** surprisingly showed very strong antibacterial and antifungal potential on the tested three Gram-positive bacteria and three yeast. **11** exhibited potent activity against C. albicans and C. parapsilosis with an MIC value of 19.53 µg/mL (Table II and Table III).

TABLE II: Minimal Inhibitory Concentrations (MICs) Values of the Tested Coumarin Derivatives for Antibacterial Activity

Compound	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 4352	*P. mirabilis* ATCC 14153	*E. faecalis* ATCC 29212	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 29213
1	-	-	-	-	625	1250	625
2	-	-	-	-	1250	-	-
3	-	-	-	-	-	-	-
4	-	625	625	-	1250	625	1250
5	-	-	-	-	1250	1250	1250
6	-	-	-	-	1250	-	-
7	-	-	-	-	1250	1250	1250
8	-	-	-	-	312.5	**156.2**	**78.12**
9	-	-	-	-	-	-	-
10	-	-	-	-	625	1250	1250
11	-	625	312.5	312.5	**78.12**	**156.2**	**156.2**
12	-	625	-	625	625	**156.2**	**156.2**
13	-	-	-	-	312.5	**78.12**	**156.2**
Reference	2.4[a]	4.9[b]	4.9[b]	2.4[b]	128[c]	9.8[d]	1.2[b]

[a] Ceftazidime [b]Cefuroxime-Na [c]Amikacin [d] Cefuroxime

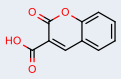
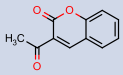
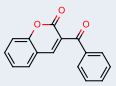
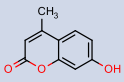
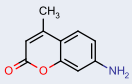
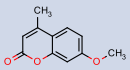
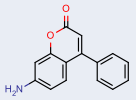
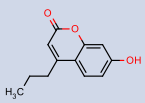
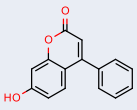
TABLE III: MINIMAL INHIBITORY CONCENTRATIONS (MICS) VALUES OF THE TESTED COUMARIN DERIVATIVES FOR ANTIFUNGAL ACTIVITY

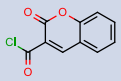
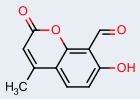
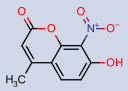
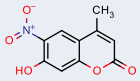
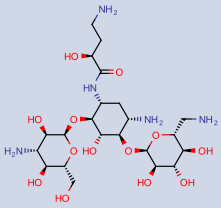
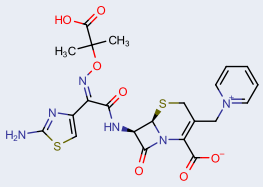
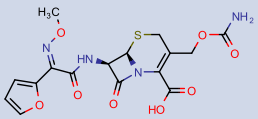
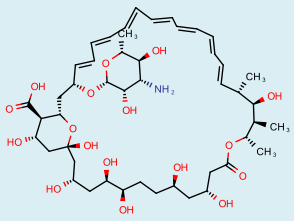
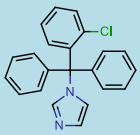
Compound	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 750
1	-	-	-
2	312.5	-	625
3	-	-	-
4	-	-	625
5	-	-	-
6	-	-	-
7	-	-	-
8	156.2	156.2	-
9	-	-	-
10	-	-	-
11	**19.53**	**19.53**	**156.2**
12	-	-	-
13	312.5	-	-
Reference	4.9[a]	0.5[b]	1[b]

B. Molecular Docking Results

Docking analyzes were performed to understand the molecular interaction mechanisms between the synthesized compounds and some selected key target proteins from S. aureus and C. albicans. Docking results of all compounds are given in Table IV, Fig. 1, and Fig. 2.

TABLE IV: DOCKING CALCULATION SCORES OF NEWLY SYNTHESIZED COMPOUNDS AND REFERENCE INHIBITORS FOR SOME SELECTED TARGET STRUCTURES

STRUCTURE	MOLECULE ID	2XCS	6CGD	4P20	5TZ1
	1	-5.16 LE: 0.37	-4.73 LE: 0.34	-4.48 LE: 0.32	-6.03 LE: 0.43
	2	-5.24 LE: 0.37	-4.99 LE: 0.36	-4.55 LE: 0.32	-6.00 LE: 0.43
	3	-6.02 LE:0.32	-5.74 LE: 0.30	-5.09 LE: 0.27	-6.70 LE: 0.35
	4	-5.09 LE: 0.39	-4.46 LE: 0.34	-4.39 LE: 0.34	-5.93 LE: 0.46
	5	-4.97 LE: 0.38	-4.57 LE: 0.35	-4.35 LE: 0.33	-6.05 LE: 0.47
	6	-5.30 LE: 0.38	-5.00 LE: 0.36	-4.60 LE: 0.33	-6.34 LE: 0.45
	7	-5.66 LE: 0.31	-5.46 LE: 0.30	-4.75 LE: 0.26	-6.62 LE: 0.37
	8	-5.35 LE: 0.36	-5.19 LE: 0.35	-4.67 LE: 0.31	-6.58 LE: 0.44
	9	-5.61 LE: 0.31	-5.46 LE: 0.30	-4.86 LE: 0.27	-6.48 LE: 036

STRUCTURE	MOLECULE ID	2XCS	6CGD	4P20	5TZ1
	10	-5.16 LE: 0.37	-4.96 LE: 0.35	-4.51 LE: 0.32	-6.10 LE: 0.44
	11	-6.35 LE: 0.42	-4.88 LE: 0.33	-4.50 LE: 0.30	-7.32 LE: 0.49
	12	-5.50 LE: 0.34	-4.94 LE: 0.31	-4.63 LE: 0.29	-6.63 LE: 0.41
	13	-5.52 LE: 0.35	-5.11 LE: 0.32	-4.67 LE: 0.29	-6.40 LE: 0.40
	amikacin	-8.94 LE: 0.22	-7.77 LE: 0.19	-7.44 LE: 0.19	-11.33 LE: 0.28
	ceftazidime	-9.09 LE: 0.25	-7.85 LE: 0.21	-7.11 LE: 0.19	11.08 LE: 0.30
	cefuroxime	-7.70 LE: 0.27	-7.16 LE: 0.25	-6.41 LE: 0.22	-9.49 LE: 0.33
	Amphotericin B	-11.94 LE: 0.18	-10.00 LE: 0.15	-10.12 LE: 0.16	-8.98 LE: 0.14
	clotrimazole	-5.76 LE: 0.23	-5.68 LE: 0.23	-4.80 LE: 0.19	-7.96 LE: 0.32

2XCS: PDB ID, S. aureus Gyrase complex with GSK299423 and DNA.
6CGD: PDB ID, Aminoglycoside Phosphotransferase (2")-Ia in complex with GMPPNP, and Amikacin
4P20: PDB ID, bacterial ribosomal decoding site complex with amikacin.
5TZ1: PDB ID, C. albicans 14-alpha demethylase (CYP51) complex with the tetrazole-based antifungal drug candidate VT1161 (VT1)
LE: Ligand Efficiency = -(Docking Score)/(The Number of Heavy Elements)
Note: Docking Calculation Score Unit: kcal/mol

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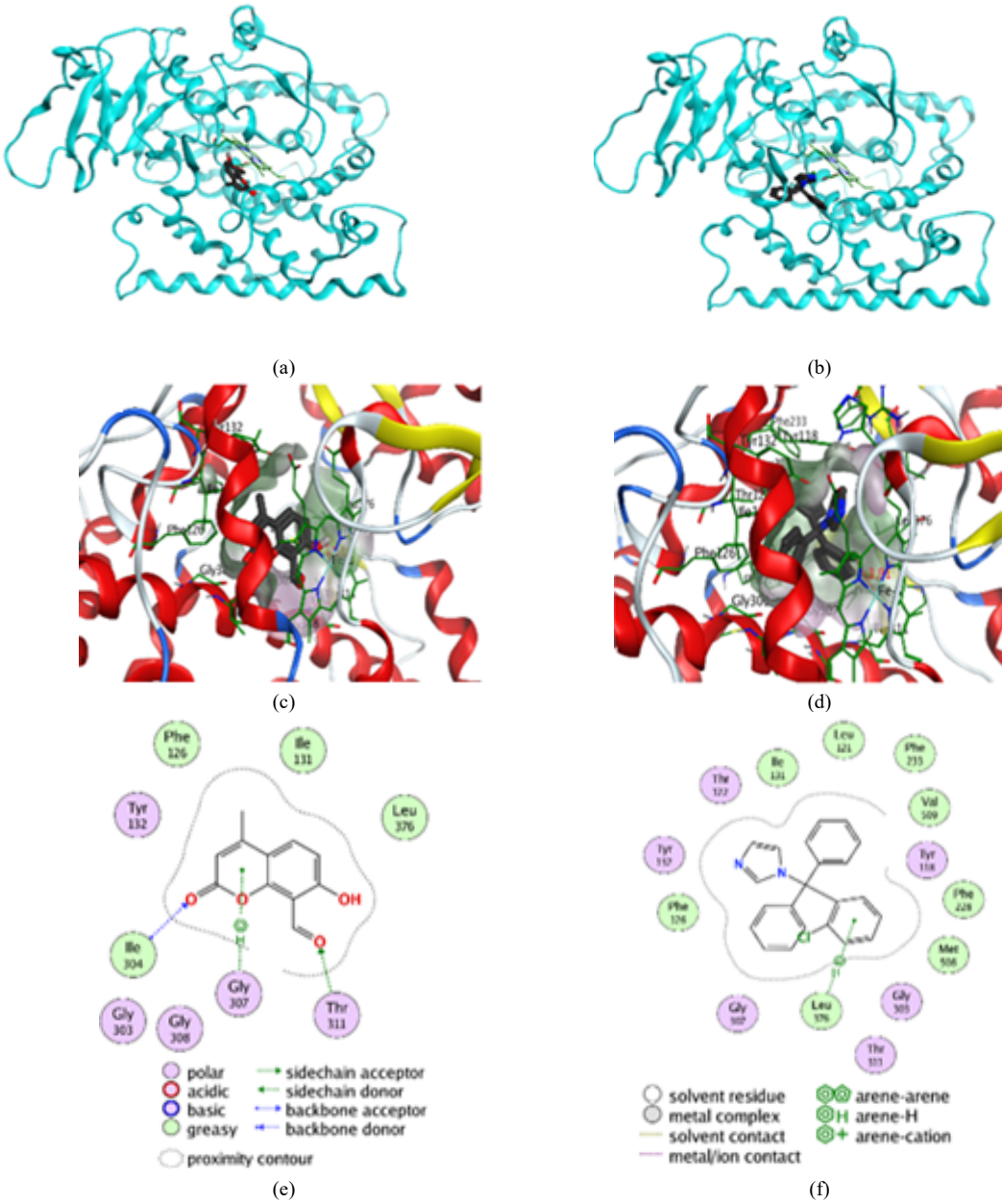

Fig. 1. Molecular docking interaction of comp.**11** and clotrimazole against CYP51 (5TZ1.PDB); (a), (c), (e) are protein-ligand interaction plots for comp.11. (b), (d), (f) are protein-ligand interaction plots for reference molecule clotrimazole.

These newly synthesized compounds failed to generate a strong binding affinity score for the selected target proteins other than CYP51 in the docking calculations, as observed in the experimental results. However, **3** and **11** were observed to have significant molecular interactions against the target structure, the S. aureus Gyrase-DNA (2XCS PDB ID) complex, despite having relatively lower docking scores. The specific protein-ligand molecular interactions of the compound **3**, compound **11** and reference molecule cefuroxime are detailed in Fig. 2.

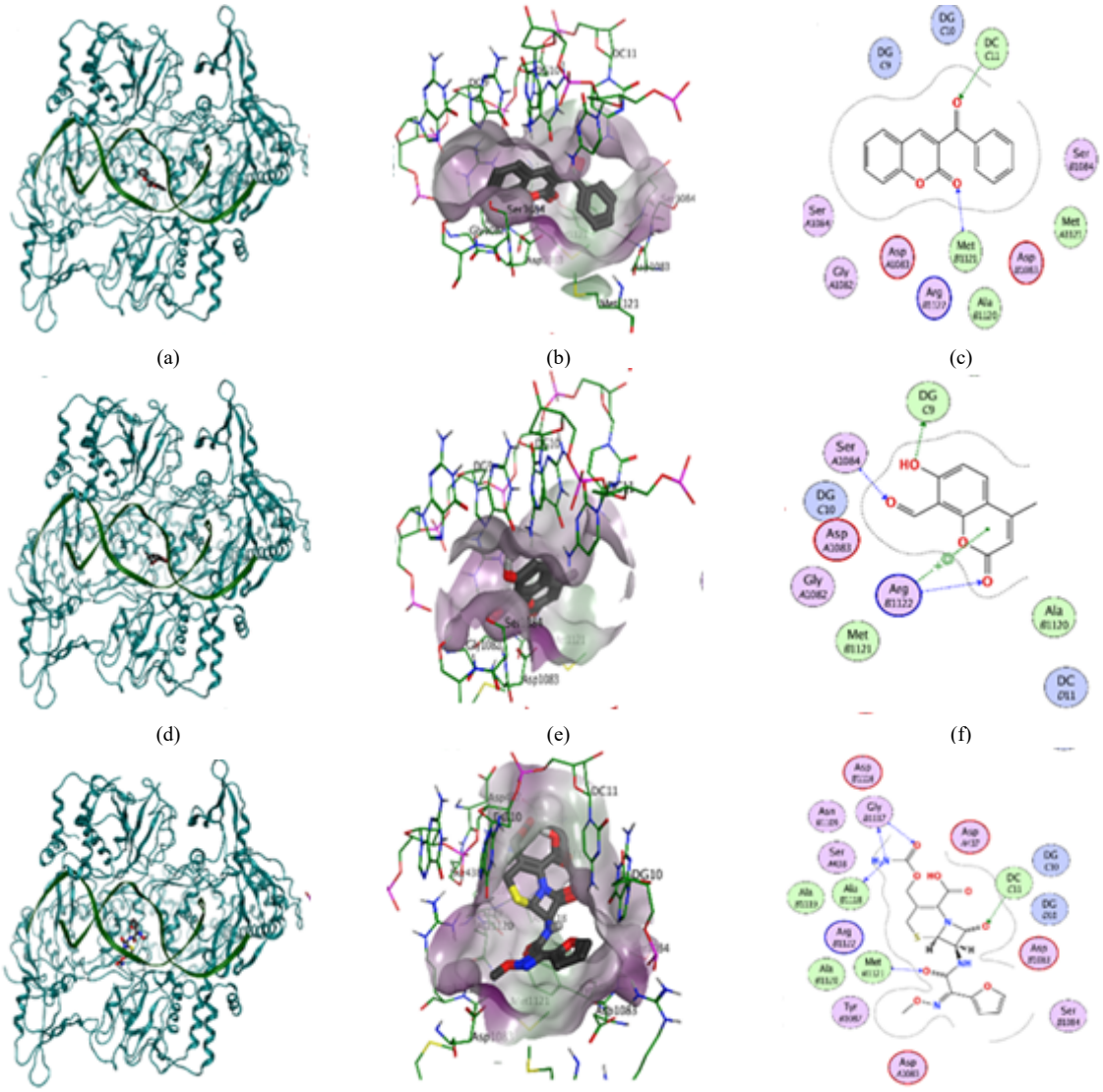

Fig. 2. Molecular docking interaction of comp.3, comp.11 and cefuroxime against Gyrase-DNA complex (2XCS.PDB). (a), (b), (c) are protein-ligand interaction plots for comp.3.; (d), (e), (f) are protein-ligand interaction plots for comp.11.; (g), (h), (i) are protein-ligand interaction plots for reference molecule cefuroxime.

IV. CONCLUSION

The in vitro antimicrobial activity of 13 coumarin derivatives were evaluated against three Gram-positive bacteria, four Gram-negative bacteria, and three fungi. The results showed that all coumarin derivatives exhibited antimicrobial activity against E. faecalis except **3** and **9**. The Compound **11** showed very strong antibacterial and antifungal potential on the tested three Gram positive bacteria and three yeast. **11** exhibited potent activity against C. albicans and C. parapsilosis with an MIC value of 19.53 µg/mL.

Based on the experimental findings of the synthesized compounds (1-13), docking analyzes were performed for some key target proteins selected from *S. aureus* and *C. albicans*. According to the results of docking calculations, the newly synthesized compounds, except **11**, could not form binding affinities as strong as the model inhibitors against selected target macromolecular structures.

11 showed high binding affinity for CYP51, which is also in agreement with the experimental results. Despite its relatively small molecular structure, **11** has a high docking score against CPY51, comparable to the model inhibitor, and strong interactions with protein residues that increase its binding stability.

This presented **11** offer an important alternative for antifungal candidate drug development studies, since they are open to advanced chemical modifications. For this purpose, **11** is open to various chemical modifications and is a good starting scaffold molecular structure for antifungal compounds designs.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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