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Preparation, Docking, Antimicrobial and Cytotoxic Activities of 2-arylquinazolinones

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NR and SK synthesized and analyzed the data of the 2-arylquinazolinones. Author KZ prepared the antifungal tests. Author Zahra Faghih prepared the cytotoxicity tests. Author Zeinab Faghih managed the Docking study. Authors SK and IY wrote the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Recently the use of antifungal drugs in human medicine has been increased, especially with the advent of AIDS epidemic. The extensive use of antifungal drugs and their resistance against fungal infections have led to the discovery of new antimicrobial compounds. Despite the growing list of azole drugs, their clinical value has been limited by their relatively high risk of toxicity and the emergence of drug resistance. Also, chemotherapy for cancer is frequently limited by organ toxicities and emergence of drug resistance in tumor cells. So efforts have focused on the development of new, less toxic, and more effective antifungal and cytotoxic agents.

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Study Design: Preparation of several 2-arylquinazolinones with both antifungal and cytotoxic activities.
Place and Duration of Study: Department of Medicinal Chemistry, Department of Medical Mycology and Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran, from October 2016 to September 2017.
Methodology: Several synthetic analogues of 2-arylquinazolinones have been developed aiming at drugs with high potency and diminished toxicity. Thus, 2-arylquinazolinones ***(1b-12b)*** are prepared according to literature. The antimicrobial activities of these compounds against different species of microorganisms including fungi, gram positive and gram negative bacteria are evaluated. Broth micro-dilution method as recommended by clinical and laboratory standard institute (CLSI) was used for this purpose. We also evaluated the cytotoxic activities of the compounds against three human cancer cell lines (MCF-7, A549 and SKOV3) using colorimetric MTT cytotoxic assay. The specific binding mode of synthetic 2-arylquinazolinones have been also indicated by molecular modelling studies to show the interactions and binding orientation of compounds to CYP51 active site.
Results: Compound ***1b*** showed desirable activities against bacteria as well as yeasts and filaments fungi. Compounds ***2b, 7b, 8b*** and ***9b*** can inhibit the growth of different yeasts with acceptable MIC. The cytotoxic results showed compounds ***7b, 8b, 9b*** and ***10b*** had partial inhibitory effects on cancer cell lines in particular lung adenocarcinoma.
Conclusion: 2-arylquinazolinones are respectable candidate for further studies in biological research.

Keywords: Quinazoline; antifungal; broth micro-dilution; cytotoxic assay.

1. INTRODUCTION

During the past decades, resistance to traditional antimicrobial drugs has enlarged affectedly. The resistant strains have important implications for morbidity, mortality, and health care costs. The emergence of multi-drug-resistant strains makes the discovery and development of new molecular scaffolds and novel classes of antimicrobials a priority to achieve effective control [1-3]. Recently azole drugs have made a significant impact on the management of fungal infections as first-choice antifungal drugs. So far a large number of azole drugs including imidazole and triazole ones have been extensively used in the treatment of various infectious diseases. However, several factors such as narrow antifungal spectrum, low bioavailability and concerns about multidrug-resistance have made most of the first-line clinical antibiotics ineffective [4,5]. This situation and the limited diversity of antimicrobial agents has stimulated an urgent medical need to develop more effective, new classes of antimicrobial agents with novel chemical structures, broad spectrum, low toxicity and low resistance which are helpful for overcoming drug-resistance and improving the antimicrobial potency [4-7].

On the other hand, several chemotherapeutic agents are currently being used to treat human cancers but they have limited success and the response rates stay mainly unimproved in clinical trials [8]. The main limitations in conventional cancer chemotherapy rise from the absence of drug-specific affinity to tumor cells and systemic toxicity leading to many negative and life-threatening side effects [9]. In spite of major advances in chemotherapeutic organization and cancer biology, the main difficulties preventing the progress of an ultimate anticancer therapy is the great likeness between tumor and normal cells. Thus, there is a critical need to discover and design new anticancer agents as well [8].

Recently synthesis of heterocyclic compounds has become one of the most essential planes of medicinal chemistry. Nitrogen-containing heterocyclic compounds are the most abundant scaffolds that occur in a variety of synthetic drugs and bioactive natural products [10]. Among the nitrogen-containing compounds the quinazoline nucleus is a very good-looking skeleton in medicinal and pharmaceutical chemistry. Quinazoline is a 1,3-diazanaphthalene which is also known as 5,6-benzopyrimidine or benzo[*a*]pyrimidine and its 4-oxo derivative is called 4(3*H*)-quinazolinone [11]. Through the 1980s quinazoline derivatives have been introduced as orally active compounds in medicine. Quinazolines are nitrogen containing heterocyclic scaffolds and have attracted significant attention due to their diverse pharmacological activities such as anticancer, antimalarial, antimicrobial, anti-inflammatory, anticonvulsant, antihypertensive, anti-diabetic, antitubercular, and anti-HIV activities [10-15].

In this regard a vast number of quinazoline derivatives have been synthesized to provide synthetic drugs and to design more effective medicines [11,16-24]. Out of the different quinazolinones categories, 2-substituted-4(3H)-quinazolinones are being studied extensively as an important pharmacophore and are most prevalent, either as natural products or pharmaceutical agents (Fig. 1) [13,14].

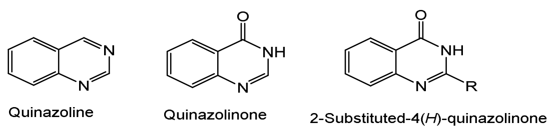


Fig. 1. Different quinazoline backbones

Recently, we also synthetized a variety of quinazoline derivatives *via* a structural modification on available quinazoline drugs [25,26]. Herein, we report on the preparation of 2-substituted-4(3H)-quinazolinones and their antibacterial, antifungal and cytotoxic activities. Broth microdilution method was used for evaluation of their antimicrobial activity against different species of candida and filaments fungi and also gram positive and gram negative bacteria. For cytotoxic activity of the quinazoline compounds, three human cancer cell lines, MCF-7 (breast carcinoma), A549 (Lung carcinoma), SKOV3 (ovarian carcinoma) and colorimetric MTT cytotoxic assay were used. In addition, docking simulation was performed to show the binding mode of these compounds to CYP51 active site.

2. MATERIALS AND METHODS

All chemicals and solvents were purchased from Merck (Germany). All yields refer to isolated products after purification methods. The progress of reactions was followed with TLC using silica gel *SILG/UV 254* plates. The products were characterized by comparison of their physical data with those of known samples.

2.1 General Procedure for Synthesis

A mixture of anthranilimide (3 mmol), $CuCl_2$ (3 mmol) and the appropriate aldehyde (3.3 mmol) in ethanol (10 mL) was refluxed for 2- 3 h. The reaction mixture was then allowed to cool to r.t., the solvent was removed in vacuo, and the crude product was purified by column chromatography over silica gel to provide pure quinazolinone in high yield (Fig. 2) [24]. The purity and justification of the final compounds were confirmed by comparing their melting points with those reported in literature (Table 1).

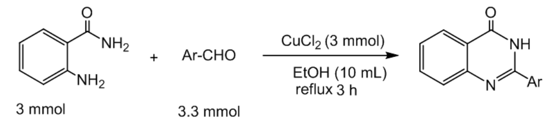


Fig. 2. Synthesis of 2-arylquinazolinones

2.2 Antimicrobial Activity

2.2.1 Microorganisms

The antifungal activities of the synthetic compounds against twelve standard strains of fungi, including *C. albicans* (ATCC 10261), *C. tropicalis* (ATCC 750), *C. glabarata* (ATCC 90030), *C. krusei* (ATCC 6258), *C. dubliniensis* (ATCC8501), *C. parapsilosis* (ATCC 4344), *C. neoformance* (ATCC 9011), *Exophiala dermatitidis* (CBS 120433), *Psuedoalscheria boydii* (CBS 329.93), *A. fumigates* (ATCC 14110), *A. flavous* (ATCC 6402) and *A. clavatus* (CBS514.65) were determined. In addition, the antibacterial activities of the synthetic compounds against standard species of *S. aureus* (ATCC 2592), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were determined in this study.

2.2.2 Determination of minimum inhibitory concentrations

The MICs were determined using the broth microdilution method recommended by the CLSI with some modifications. Briefly, for determination of antifungal activities, serial dilutions of the synthetic compounds (0.5-256 µl/ml) were prepared in 96-well microtitre plates, using Roswell Park Memorial Institute (RPMI-1640) media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). To determine the antibacterial activities, serial dilutions of the compounds (0.5-256 µl/ml) were prepared in the Muller-Hinton media (Merck, Darmstadt, Germany). For yeasts and bacteria, stock inoculums were prepared by suspending three colonies of the examined microorganisms in 5ml sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standard at 630nm wavelength (this yields stock suspension of $1\text{-}5\times10^6$ cells/ml for fungi and $1\text{-}1.5\times10^8$ cells/ml for bacteria). The working suspension was prepared by making a 1/1000 dilution of the stock suspension with RPMI or the Muller-Hinton broth for yeasts and bacteria, respectively. For molds (*Aspergillus* spp, *Psuedoalscheria*), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with Tween 20. The collected conidia were transferred in sterile saline and their turbidity was adjusted to optical density of 0.09 to 0.11 that yields $0.4\text{-}5\times10^6$ conidia/ml. The working suspension was prepared by making a 1/50 dilution with RPMI of the stock suspension. To each well of the microtiter plates, 0.1ml of the working inoculums was added and the plates were incubated in a humid atmosphere at 30ºC for 24-48 h (fungi) or at 37ºC for 24 h (bacteria). Two hundred microliters (200 µl) of un-inoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without the compounds) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥95% growth reduction compared with the growth control wells. Each experiment was performed in triplicate.

In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on the Muller-Hinton agar (Merck, Darmstadt, Germany), to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC), respectively. MFCs and MBCs were determined as the lowest concentration yielding no more than 4 colonies, which corresponds to a mortality of 98% of microorganisms in the initial inoculums.

2.3 Cytotoxic Activity

2.3.1 Cell line and cell culture

Three human cancer cell lines, MCF-7 (breast carcinoma), A549 (lung non-small cell carcinoma), SKOV3 (ovarian carcinoma) were purchased from the national cell bank of Pasteur Institute of Iran (Tehran, Iran). Under aseptic conditions, the cells were cultured in RPMI 1640 medium, containing 10% Fetal Bovine Serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (all from Biosera, France), and incubated at 37°C in a CO_2 incubator with humidified atmosphere. After 80% confluency, the cells were sub-cultured using 25% trypsin-EDTA solution (Biosera, UK) and were then counted and prepared for MTT assay.

2.3.2 MTT assay

The cytotoxic effect of compounds was determined by using a standard MTT assay as described before [27,28]. Briefly, the cells were plated at the 10×10^3 density in 100 µl per well as determined in our previous studies [29]. The cells were incubated for 24 hours to recover and reattach and were then treated with different concentrations of each compounds (10-1000 µM). Three wells were left without treatment as cell-based negative controls, and three wells of cell culture medium alone were considered as blanks. Following 48-hour incubation, the culture media were completely removed and 100 µl of MTT solution with 0.5mg/ml concentration were added to the wells including controls. The plate was incubated for 3-4 hours at 37°C and checked periodically for the appearance of purple precipitate. Then, after complete removing of MTT solution, 100 µl of DMSO was added to the wells and leaved in the dark at room temperature for more 30 min. The absorbance of all wells including the blanks, were measured at 490 nm. Each experiment was separately repeated three times.

2.4 Data Analysis

Excel 2013 was used for calculation. The average values from triplicate readings were determined and subtracted the average value for the blank. The inhibitory concentration (IC) of each compound was calculated and reported using following formula:

$$IC = 100 – [(OD_{test} – OD_{blank}) / (OD_{negative}) \times 100]$$

For each chemical a plot of the IC versus concentration was depicted using Curve Expert 1.5 software and an Inhibition Concentration 50 (IC_{50}), indicating the 50% growth inhibition of the cells was obtained for each compound.

2.5 Docking Procedure

An in house batch script (DOCK-FACE) for automatic running of AutoDock 4.2 was used to perform the docking simulations [30] in a parallel mode, using all system resources as previously described [31]. Crystal structure of Cytochrome P450 14-Alpha -Sterol Demethylase (CYP51) in complex with (PDB ID: 1EA1) was obtained from Protein Data Bank (PDB data base; http://www.rcsb.org) [32]. Co-crystal ligand and water molecules were removed to prepare the protein structure. Missing atom types of the PDB were checked by MODELLER 9.17 [33].

The ligand structures were made by using HyperChem software package (Version 7, Hypercube Inc). Molecular Mechanic (MM+), followed by semiempirical AM1 method was achieved for geometry optimization. The prepared Ligands were given to 100 independent genetic algorithm (GA) runs. 150 population size, a maximum number of 2,500,000 energy evaluations and 27,000 maximum generations were used for Lamarckian GA method. The grid points of 40, 40, and 40 in x-, y-, and z directions were used. A grid spacing of 0.375 A° was built centered on Hem group in the catalytic site of the receptor. Number of points in x, y and z was -17, -3 and 65 respectively. All visualization of protein ligand interaction was evaluated using VMD software [30].

3. RESULTS AND DISCUSSION

3.1 Chemistry

A plausible mechanism for the reaction between aldehyde and anthranilamide is shown in Fig. 3.

Twelve derivatives of 2-arylquinazolinones were prepared in desirable yield. Proof of identity of the final products was checked by comparing their melting points with those reported in literature [34-41]. Different characteristic of the synthesized compounds are showed in Table 1.

3.2 Antimicrobial Activities

Antifungal activities of the tested compounds against yeasts and filamentous fungi are presented in Table 2 and Table 3 respectively. As to the results, compounds ***8b*** exhibited antifungal activity against all tested Candida species except *C. krusei* at concentration of 128-256μg/ml. Moreover, compound ***2b*** and ***7b*** inhibited growth of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis* and *Psuedoalscheria boydii* at concentration rate of 32-256/ml. In addition the growth of *C. neoformance* was inhibited by compounds ***1b, 6b*** and ***8b*** at concentration of 64-256 μg/ml.

As shown in Table 3, compounds ***1b, 2b, 7b, 8b*** and ***9b*** exhibited inhibitory activity against some of the *Aspergillus* species at concentration of 128-256 μg/ml. Of the synthetic compounds only compounds ***1b*** and ***8b*** successfully inhibited the growth of black fungi, *Exophia dermatitis*, at concentration of 256 μg/ml and 128 μg/ml, respectively.

Antibacterial activities of the synthetic compounds against gram positive and gram negative bacteria are shown in Table 4. Of the synthetic compounds, ***1b*** and ***9b*** inhibited the growth of *S. areus* at concentration of 256 μg/ml. Compounds ***1b, 2b, 4b*** showed inhibitory effect on *E. coli* at concentrations ranging from 128-256 μg/ml.

3.3 Cytotoxic Activities

Three cancer cell lines MCF-7 (breast carcinoma), A549 (lung carcinoma) and SKOV3 (ovarian carcinoma) were used to evaluate the cytotoxicity effects of quinazoline compounds on tumor cells. As demonstrated in Table 5, compounds ***7b***, ***8b***, ***9b*** and ***10b*** have moderate cytotoxic effects on different tumor types ($IC_{50<}$1000). Better cytotoxic activities could be observed on lung carcinoma, A549 (IC_{50} from 176 to 388). Breast carcinoma, MCF-7, is in the second place (IC_{50} from 385 to 650) and less effects were observed on ovarian cancer, SKOV3, (IC_{50} from 785 to 900).

3.4 Docking Studies

To find the binding orientation of the 2-arylquinazolinones to their protein targets, docking method was used. It was also applied for prediction and elucidation of the compounds` affinity and activity according to their binding energy. Following the docking procedure, the protein–ligand complex was studied to find the type of interactions. All the docking protocols were performed on validated structures, with RMSD values below 2Å. The conformation with the lowest docking binding energies was considered as the best docking result. These energies were summarized in Table 6.

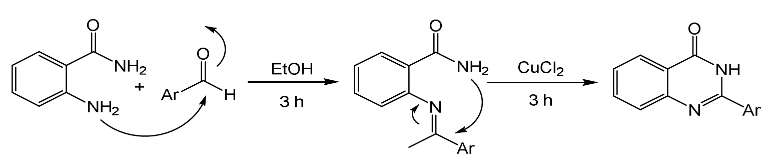
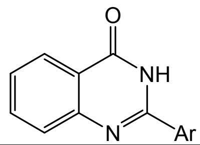
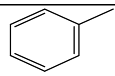
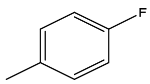
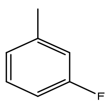
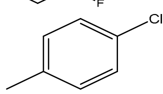
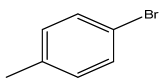
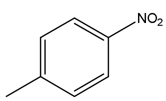
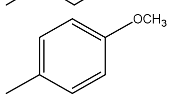
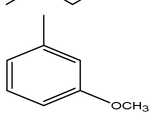
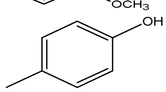
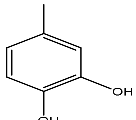
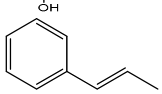
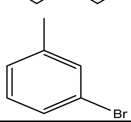


Fig. 3. A plausible mechanism for the formation of 2-arylquinazolinones

Table 1. Synthesized 2-arylquinazolinones which were tested against fungi and bacteria



Entry	Ar	Chemical Names (M.W.)	M.P.(˚C) (Reported)	M.P.(˚c) (Found)	Yield (%)
1b		2-phenyl-3*H*-quinazolin-4-one (222.24)	232-235	231-234	65
2b		2-(4-Fluoro-phenyl)-3*H*-quinazolin-4-one (240.23)	262-265	260-263	84
3b		2-(3-Fluoro-phenyl)-3*H*-quinazolin-4-one (240.23)	261-263	259-261	92
4b		2-(4-Chloro-phenyl)-3*H*-quinazolin-4-one (256.69)	298-300	298-300	91
5b		2-(4-Bromo-phenyl)-3*H*-quinazolin-4-one (301.14)	291-294	291-294	93
6b		2-(4-Nitro-phenyl)-3*H*-quinazolin-4-one (267.24)	358-360	355-360	64
7b		2-(4-Methoxy -phenyl)-3*H*-quinazolin-4-one (252.09)	245-247	244-248	60
8b		2-(3-Methoxy -phenyl)-3*H*-quinazolin-4-one (252.09)	195-197	194-198	66
9b		2-(4-Hydroxy -phenyl)-3*H*-quinazolin-4-one (238.24)	300	296-298	52
10b		2-(3,4-Dihydroxy -phenyl)-3*H*-quinazolin-4-one (254.24)	230	296-300	54
11b		2-Styryl-3*H*-quinazolin-4-one (248.28)	238-240	240-243	70
12b		2-(3-Bromo-phenyl)-3*H*-quinazolin-4-one (301.14)	295-296	288-291	93

As displayed in Table 6, all investigated complexes showed better docking binding energies than the co-crystal ligands (fluconazole). The interaction modes of ***1b, 2b, 8b*** and ***9b***, those with the appropriate antimicrobial activities are shown in Fig. 4.

The docking model indicated the specific alignment of quinazoline ring to the pocket of heme iron of CYP51 in these two ligands. This orientation with respect to the hydrogen bonding and hydrophobic interactions may be in favor of antimicrobial activity.

Table 2. MIC values (µg/ml) for tested compounds against different yeasts

Entry	*C. albicans*		*C. tropicalis*		*C. glabrata*		*C. krusei*		*C. dubliniensis*		*C. parapsilosis*		*C. neoformans*	
	MIC90	**MFC**	**MIC90**	**MFC**	**MIC90**	**MFC**	**MIC90**	**MFC**	**MIC90**	**MFC**	**MIC90**	**MFC**	**MIC90**	**MFC**
1b	256	G	128	G	256	G	256	G	128	G	128	G	256	G
2b	128	G	256	G	G	G	G	G	128	G	32	64	G	G
3b	G	G	G	G	G	G	G	G	G	G	G	G	G	G
4b	G	G	64	128	G	G	G	G	G	G	G	G	G	G
5b	G	G	G	G	G	G	G	G	G	G	G	G	G	G
6b	G	G	G	G	G	G	G	G	G	G	G	G	64	128
7b	256	G	128	256	G	G	G	G	256	G	128	256	G	G
8b	256	G	256	G	256	G	G	G	128	G	256	G	256	G
9b	128	G	128	G	G	G	256	G	256	G	64	128	G	G
10b	G	G	G	G	G	G	G	G	G	G	G	G	G	G
Fluconazole	4	ND	2	ND	1	ND	64	ND	0.25	ND	0.25	ND	0.25	ND

G: Concentration >256 µg/ml, ND: Not determined

Table 3. MIC values (µg/ml) for tested compounds against different filamentous fungi

Entry	*A. flavus*		*A. clavatus*		*A. fumigatus*		*Exophiala*		*P. boydii*	
	MIC90	MFC	MIC90	MFC	MIC90	MFC	MIC90	MFC	MIC90	MFC
1b	256	G	G	G	G	G	256	G	128	G
2b	G	G	G	G	G	G	G	G	256	G
3b	G	G	G	G	G	G	G	G	G	G
4b	G	G	G	G	G	G	G	G	G	G
5b	G	G	G	G	G	G	G	G	G	G
6b	G	G	G	G	G	G	G	G	G	G
7b	G	G	128	G	G	G	G	G	128	G
8b	256	G	256	G	G	G	128	G	256	G
9b	G	G	G	G	256	G	G	G	G	G
10b	G	G	G	G	G	G	G	G	G	G
Fluconazole	**16**	**ND**	**8**	**ND**	**8**	**ND**	**2**	**ND**	**4**	**ND**

G: Concentration >256 µg/ml; ND: Not determined

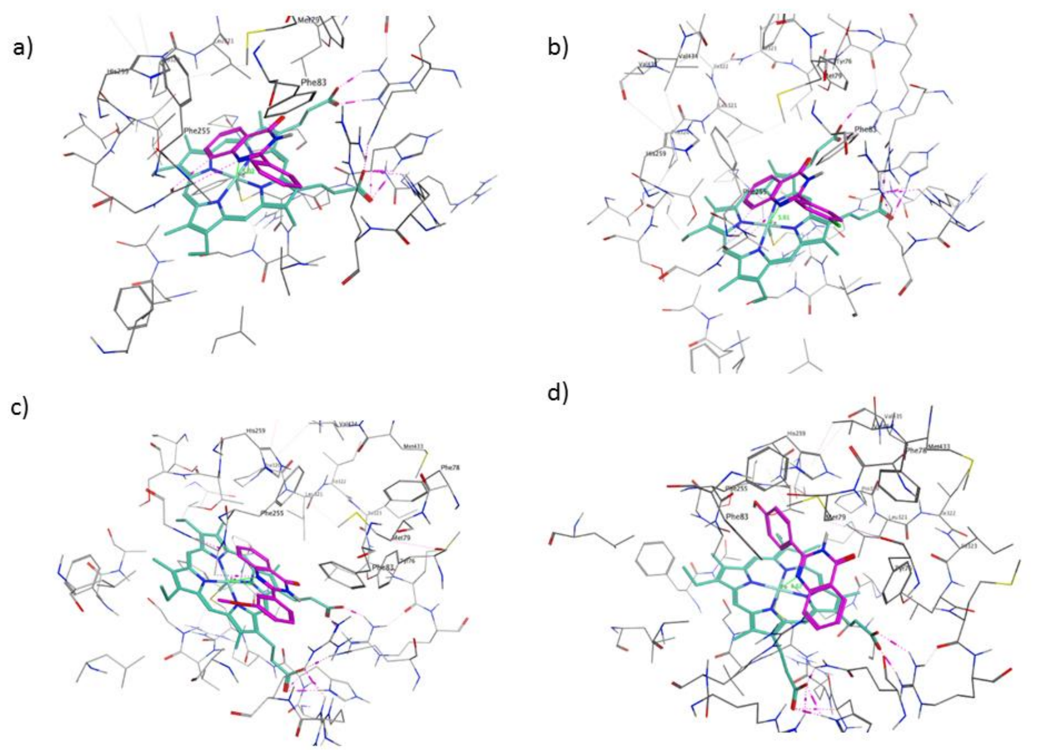


Fig. 4. The docked configuration of *1b* (a), *2b (b)*, *8b (c)* and *9b (d)* in the binding site of lanosterol 14-α-demethylase (CYP51)

Table 4. MIC values (µg/ml) for tested compounds against different bacteria

Entry	*S. aureus*		*E. coli*		*P. aeruginosa*	
	MIC90	MBC	MIC90	MBC	MIC90	MBC
1b	256	G	128	G	G	G
2b	G	G	256	G	G	G
3b	G	G	G	G	G	G
4b	G	G	128	G	G	G

Entry	*S. aureus*		*E. coli*		*P. aeruginosa*	
	MIC90	MBC	MIC90	MBC	MIC90	MBC
5b	G	G	G	G	G	G
6b	G	G	G	G	G	G
7b	G	G	G	G	G	G
8b	G	G	G	G	G	G
9b	256	G	G	G	G	G
10b	G	G	G	G	G	G
Ciprofloxacin	0.5	G	0. 025	G	2	G

G: Concentration >256 µg/ml; ND: Not determined

Table 5. In vitro cytotoxicity effect of 2-arylquinazolinones on cancer cell lines

Compounds	IC_{50} (µM)		
	MCF-7	A549	SKOV3
1b	>1000	>1000	>1000
2b	>1000	>1000	>1000
3b	>1000	>1000	>1000
4b	>1000	>1000	>1000
5b	>1000	>1000	>1000
6b	>1000	>1000	>1000
7b	412	210	846
8b	650	388	840
9b	385	264	900
10b	485	176	785
Cis-platin	61	50.81	43.81

Table 6. Molecular docking Studies on the CYP51 active site

Entry	Binding Energy (Kcal/mol)[a]
1b	-7.76
2b	-7.21
3b	-7.73
4b	-7.56
5b	-7.83
6b	-7.73
7b	-7.73
8b	-8.01
9b	-7.64
10b	-7.76
Fluconazol	-6.47

[a] All the docking protocols were performed on validated structures with RMSD values below 2 Å

4. CONCLUSION

Our results indicate that replacement of an aryl hydrogen atom of 2-arylquinazolinones with a halogen group, reduces their antifungal activity as compared to the base compound (***1b***). By comparing the molecular structures of compounds ***2b*** with ***3b, 7b*** with ***8b*** and ***9b*** with ***10b***, it seems that replacement of substitutions from meta position to para position of the phenyl ring the biological activities increased. In comparing MIC values of the basic compounds ***1b*** with those of ***2b, 4b*** and ***9b*** against the examined bacteria, it seems that replacements of hydrogen in phenyl ring with chlorine atom is more favorite for antimicrobial activities. Docking method was used to show the interactions and binding mode of these compounds to CYP51 active site. All investigated compounds showed better docking binding energies than the fluconazole.

According to the cytotoxic results compounds ***7b-10b*** showed upgraded potency for cytotoxic activity. So it seems that methoxy or hydroxyl groups are more suitable for this purpose.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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