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Original Article

Synthesis, Antimicrobial Activity and Molecular Docking Study of Novel N,2-Diphenylquinazolin-4-amine Derivatives

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Abstract

A new series of derivatives of *N*, 2-diphenylquinazolin-4-amine (3a-g) was synthesized through nucleophilic substitution. The structures of compounds were characterized by FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. All synthesized compounds were evaluated for their antimicrobial activities against Gram-positive *(Staphylococcus aureus, Bacillus subtilis, Lactobacillus rhamnosus)* and Gram-negative *(Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and also for antifungal activities, against *Candida albicans*, using broth microdilution method to determine their minimum inhibitory concentrations (MIC). Most of the compounds have shown moderate to good antibacterial activities, significantly compound 3g at 0.0625 mg/mL concentration had the highest activity against *P. aeruginosa*. Also, the MIC of compound 3f was 0.0078 mg/mL against *S. aureus*. Furthermore, the tested compounds exhibited remarkable antifungal activities against *C. albicans*, significantly compounds 3c and 3g showed the least MIC (equal to 0.0625 mg/mL). Also, a docking study into DNA gyrase has been made for these compounds. The synthesized compounds showed dock score values between -3.05 and -6.13kcal/mol. The highest dock score among them was -6.13 kcal/mol, found for compound 3c.

Keywords: Synthesis; Quinazoline; Molecular docking; Antimicrobial activity; DNA gyrase; Gram-positive bacteria; Gram-negative bacteria.

1. Introduction

Nowadays, antibacterial resistance is recognized as a significant threat to health worldwide. Therefore, the search for new antibacterial drug compounds is an attractive goal for medicinal chemists [1- 4]. Quinazoline heterocycles are functional bioactive scaffolds in medicinal chemistry. The simple and condensed quinazoline derivatives possess diverse pharmacological activities, including anticancer [5], antihistaminic [6], antinociceptive [7], antithrombotic [8], anticonvulsant [9], and anti-inflammatory [10]. Quinazolines are well

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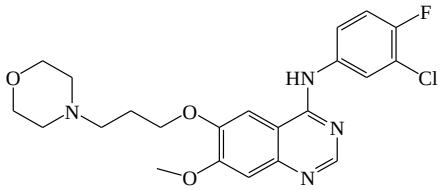
known to play a significant role as inhibitors. An example is the 4-anilinoquinazoline used as an epidermal growth factor receptor (EGFR) inhibitor. Gefitinib (ZD-1839, Iressa) and erlotinib (OSI-774, Tarceva) are used as dual EGFR- human epidermal growth factor receptor 2 (HER2) inhibitors, which are used in the clinic (**Scheme 1**) [11].

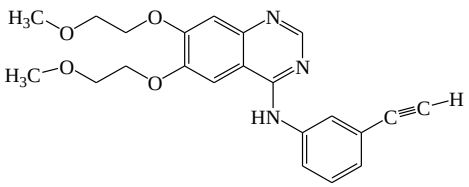
Among various pharmacological activities, quinazoline derivatives have significant antimicrobial properties. DNA gyrase is one of the interesting targets in *Escherichia coli* that catalyze changes in the topology of DNA and induces the formation of negative supercoils [12]. Due to its vital role in the survival of bacterial cells and the lack of its existence in higher eukaryotes, bacterial DNA gyrase has been used as an antibacterial target.

Molecular docking is used to predict the interactions between a ligand and a receptor molecule to predict ligand conformation and orientation within a targeted binding site [13, 14].

In the present work, we have synthesized some new derivatives of *N*, 2-diphenylquinazolin-4-amine containing phenyl group at position 2, and various aniline derivatives at the 4th position of the quinazoline ring (**Scheme 2**). In addition, the antimicrobial activities of all synthesized *N*, 2-diphenylquinazolin-4-amine derivatives were evaluated against both Gram-positive and Gram-negative bacteria as well as fungal strains. Among the compounds tested, some of *N*, 2-diphenylquinazolin-4-amine were found to be superior in inhibiting the growth of all the bacterial and fungal strains. The synthesized derivatives were docked into the binding pocket of DNA gyrase protein, and their binding energies were calculated.

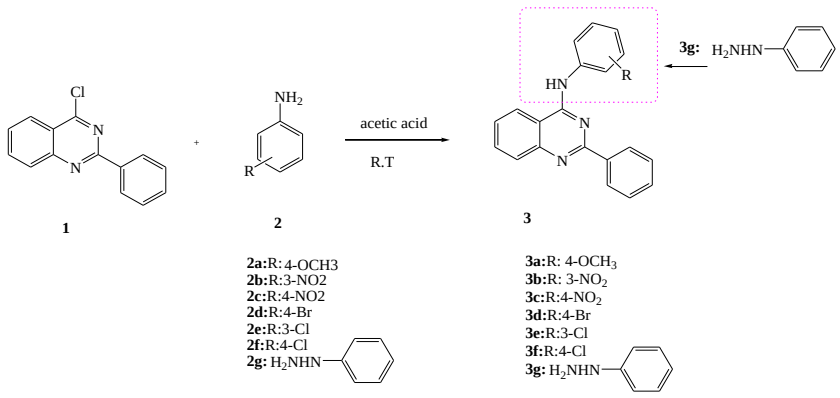


Gefitinib



Erlotinib

Scheme 1. Chemical structure of some epidermal growth factor receptor tyrosine kinase inhibitors [4].



Scheme 2. General reaction schemes for the synthesis of the target compounds 3a-g.

2. Materials and Methods

2.1. Chemistry

All required commercially available reagents and solvents used in this study were purchased from Merck Company (Germany). The reactions were followed by thin-layer chromatography on silica gel (F245 Merck plates, Merk, Germany). Melting points of the synthesized compounds were determined in open capillaries using the Electrothermal 9300 apparatus. IR spectra were recorded on an FT-IR Nicolet 4700 FT-IR spectrophotometer.^{1}H and ^{13}C NMR spectra were obtained on a Bruker-Instrument DPX-400 Avance, operating at 400 MHz for ^{1}H, and 100MHz for ^{13}C, respectively.

2.2. Antibacterial activity

The *in vitro* antimicrobial evaluation of the synthesized compounds **(**3a-g**)** was carried out using the broth microdilution method in a 96-well microtiter plate against three Gram (+) bacteria (*Bacillus subtilis* ATCC 6633, *Lactobacillus rhamnosus* ATCC 7469, and *Staphylococcus aureus* ATCC 25923) and two Gram (-) bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853*)* as well as one fungus (*Candida albicans* ATCC 10231). Ciprofloxacin (Exir, Iran) and nystatin (Farabi, Iran) were taken as standard drugs for antibacterial and antifungal activity, respectively. Microorganisms that were used in the antibacterial evaluation were purchased from the Persian Type Culture Collection (Iran). Muller Hinton medium and Sabouraud dextrose medium were purchased from QLab (USA).

2.3. Molecular docking

Hardware and Software: All the molecular modeling studies described herein were performed on ASUS Laptop (Intel® Core™i7-7500T CPU @ 2.70 GHz, RAM 8 GB) running Windows 10 64-bit HomeBasic Operating System. For *in silico* protein-ligand docking simulation, AutoDock 4.2, Hyper Chem 7.0, and Discovery Studio 2.5 software packages were used.

2.4. Chemistry

The designed compounds were prepared through the nucleophilic substitution reaction of substituted anilines with 4-chloro-2-phenylquinazoline (1) and characterized by spectroscopic methods.

2.4.1. General Procedure for the Synthesis of Target Compounds (3a-g)

1 mmol of 4-chloro-2-phenylquinazoline was taken in a 50 ml round bottom flask, equipped with an ice bath and overhead stirrer, and 1 mmol of substituted aniline was added to the flask, then acetic acid (10 ml) was added to the flask. This solution was stirred for an appropriate time. After completing the reaction as indicated by TLC, the precipitate was collected by filtration, recrystallized in ethanol, and characterized by different techniques.

2.4.2. Synthesis of N-(4-methoxyphenyl)-2-phenylquinazolin-4-amine(3a)

Yellow-slightly White solid, yield: 73 %, M.p: 265-266 °C. FT-IR (KBr cm^{-1}): 3329.7

(N-H), 3187.48 (C-H arom, str.), 2988.38 (C-H aliph, str.), 1599.7 (C=N, str.). ^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.7(1H, d, H-Qu), 8.6(1H, d, H-Qu), 8.4(1H, t, H-Qu), 8.2(1H, t, H-Qu), 7.6-7.9(5H, m, H-Ar), 7.4(1H, d, H-Ar), 7.3(1H, d, H-Ar), 7.2(1H, d, H-Ar), 7.1(1H, d, H-Ar), 4.3 (1H, s, NH-Ph), 2.0 (s, 3H, OCH_3).

2.4.3. Synthesis of N-(3-nitrophenyl)-2-phenylquinazolin-4-amine(3b)

Yellow-slightly White solid, yield: 75%. M.p: 285-2860 °C. FT-IR (KBr cm^{-1}): 3384.56 (N-H), 3096.15 (C-H arom, str.), 1620.27 (C=N, str.), 1550.24 (NO2) ^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.8(1H, d, H-Qu), 8.6(1H, d, H-Qu), 8.4(1H, t, H-Qu), 8.2(1H, t, H-Qu), 7.6-7.9(5H, m, H-Ar), 7.5(1H, d, H-Ar), 7.3(1H, d, H-Ar), 7.1(1H, t, H-Ar), 7.0(1H, s, H-Ar), 4.1 (1H, s, NH-Ph.).

2.4.4. Synthesis of N-(4-nitrophenyl)-2-phenylquinazolin-4-amine(3c)

White-Yellow solid, yield: 70 % M.p: 280-281 °C. FT-IR (KBr cm^{-1}): 3164.33 (N-H), 2992.13 (C-H arom, str.), 1670.27 (C=N, str.), 1530.24 (NO2). ^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.6(1H, d, H-Qu), 8.5(1H, d, H-Qu), 8.4(1H, t, H-Qu), 8.3(1H, t, H-Qu), 7.7-7.9(5H, m, H-Ar), 7.5(1H, d, H-Ar), 7.4(1H, d, H-Ar), 7.2(1H, d, H-Ar), 7.0(1H, d, H-Ar), 4.3 (1H, s, NH-Ph.).

2.4.5. Synthesis of N-(4-bromophenyl)-2-phenyl quinazoline-4-amine(3d)

White-Yellow solid, yield: 91 %, M.p: 286-287 °C. FT-IR (KBr cm^{-1}): 3319.7 (N-H),

3301.1 (C-H arom, str.), 1599.7 (C=N, str.), 687.85 (C-Br).

^{1}H NMR (DMSO-*d6*, 400 MHz)δ /ppm: 8.9(1H, d, H-Qu), 8.8(1H, d, H-Qu), 8.6(1H, t, H-Qu), 8.4(1H, t, H-Qu), 8.2(2H, d, H-Ar), 8.0(2H, d, H-Ar), 7.6-7.8(5H, m, H-Ar), 4.0 (1H, s, NHPh).^{13}CNMR (DMSO-*d6*, 100 MHz) δ/ppm: 159.5, 157.2, 155.1, 148.1, 147.2, 144.5, 140.2, 138.4, 137.3, 132.4, 129.6, 127.4, 125.5, 120.3, 116.4, 113.4, 110.4, 108.8, 76.1, 73.3.

2.4.6. Synthesis of N-(3-chlorophenyl)-phenyl quinazoline in-4-amine (3e)

White-Yellow solid, yield: 82 %, M.p: 265-266 °C. FT-IR (KBr cm^{-1}): 3323.99 (N-H), 2389.73 (C-H arom, str.), 1599.81 (C=N, str.), 768.85 (C-Cl).

^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.8(1H, d, H-Qu), 8.5(1H, d, H-Qu), 8.3(1H, t, H-Qu), 8.2(1H, t, H-Qu), 7.8-7.9(5H, m, H-Ar), 7.7(1H, d, H-Ar), 7.6(1H, d, H-Ar), 7.5(1H, t, H-Ar), 7.4(1H, s, H-Ar), 4.1 (1H, s, NH-Ph).

^{13}CNMR (DMSO-*d6*, 100 MHz) δ/ppm: 159.1, 158.2, 157.1, 145.1, 144.2, 143.5, 139.2, 136.4, 134.3, 131.4, 128.6, 126.4, 121.5, 118.3, 115.4, 112.4, 109.4, 107.8, 77.1, 75.3.

2.4.7. Synthesis of N-(4-chlorophenyl)-2-phenyl quinazoline-4-amine(3f)

White-Yellow solid, yield: 87 %, M.p: 270-271 °C. FT-IR (KBr cm^{-1}): 3477.03 (N-H), 3071.08 (C-H arom, str.), 1677.38 (C=N, str.), 788.85 (C-Cl).

^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.8(1H, d, H-Qu), 8.5(1H, d, H-Qu), 8.4(1H, t, H-Qu), 8.3(1H, t, H-Qu), 8.1(2H, d, H-Ar), 7.8(2H, d, H-Ar), 7.6-7.4(5H, m, H-Ar), 4.2 (1H, s, NH-Ph).

^{13}CNMR (DMSO-*d6*, 100 MHz) δ/ppm: 159.4, 157.6, 156.2, 146.4, 145.6, 142.3, 138.5, 135.7, 133.3, 130.4, 127.6, 125.4, 120.5, 119.1, 116.4, 113.5, 108.4, 107.3, 78.1, 74.3.

2.4.8. Synthesis of N-(2-phenylquinazolin-4-yl) phenyl hydrazine(3g)

Yellow-slightly White solid, yield: 72 %, M.p: 220-221 °C. FT-IR (KBr cm^{-1}): 3419.7 (N-H), 3166.26 (C-H arom, str.), 1615.45 (C=N, str.). ^{1}H NMR (DMSO-*d6*, 400 MHz)*δ* /ppm: 8.9(1H, d, H-Qu), 8.7(1H, d, H-Qu), 8.2(1H, t, H-Qu), 8.1(1H, t, H-Qu), 7.7-7.9(5H, m, H-Ar), 7.6(1H, d, H-Ar), 7.5(1H, d, H-Ar), 7.4(1H, d, H-Ar), 7.2(1H, d, H-Ar), 4.0(1H, s, NH-Ph), 4.1(1H, s, NH-Ph). ^{13}CNMR (DMSO-*d6*, 100 MHz) δ/ppm: 158.1, 157.2, 155.1, 147.1, 146.2, 144.5, 137.2, 135.4, 133.3, 130.4, 129.6, 125.4, 120.5, 117.3, 114.4, 111.4, 108.4, 106.8, 76.1, 73.3.

2.5. Molecular Docking

Docking experiments were performed using AutoDock 4.2. Software [15]. The crystal structure of the DNA gyrase (PDB code 1KZN) with resolution 2.3 Å was imported into AutoDock [16]. Ligand structures were optimized using HyperChem 7.0 software (version 7.0; Hypercube, Inc., Gainesville, FL, USA; http://www.hyper.com) as reported in previous studies [17]. The Docking studies were performed according to the reported methods [18]. A grid box with the dimension of 46 × 46 × 46 Å centered on 19.259, 29.159, and 42.461 was created around the binding site of DNA gyrase protein using ADT. A Lamarckian genetic algorithm (LGA) program was used to calculate 100 different conformers (Table 1) [19].

2.6. Antibacterial Activity

The antimicrobial effect of the synthesized compounds against *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *L. rhamnosus*, and *C. albicans* was assessed by the microdilution method [20-23]. Separately, 200 µL aliquot of the stock solution of the tested compound in M-H broth for bacteria and SD broth for fungi were added to the first well of each row in a 96-well plastic plate and serially diluted in each horizontal row with 100 µL of the culture medium. Then, 100 µL of bacterial suspension (1×10^6 CFU/mL) and 15 µL of resazurin sodium solution were transferred to each well. The total volume of each well was 215. The tested compound-free medium containing bacterial suspension and the sterile medium was used as non-treated control and blank, respectively. Following 24 h incubation at 35°C for bacteria and 48 h at 25 °C for fungi, the wells were tested for the visible color change to pink, indicating bacterial growth. The lowest tested compound concentration in which the resazurin sodium did not show any visible color change was considered as minimum inhibitory concentration (MIC).

Then the content of wells without color change was transferred onto the M-H agar plates for bacteria and SD agar plates for fungi. Following 24 h incubation at 35 °C for bacteria and 48 h at 25 °C for fungi, the plates were checked for colonies' growth. The lowest tested compound concentration was not detected and was considered as minimum bactericidal concentration (MBC) (Tables 2 and 3) [24, 25].

3. Results and Discussion

3.1. Chemistry

The designed compounds were synthesized through the S_NA_r reaction of substituted anilines

with 4-chloro-2-phenylquinazoline (1) as presented in Scheme 2. The reaction was done by the nucleophilic attack of NH_2 to the fourth position of the quinazoline ring to displace the chlorine moiety [26, 27].

3.2. Molecular Docking Studies

To explore the binding modes of the newly synthesized *N*,2-diphenylquinazolin-4-amine derivatives (3a-g) with the active site of *E. coli* DNA gyrase, a molecular docking simulation was accomplished using AutoDock 4.2. software. Firstly, chloropicrin (the original co-crystallized ligand) was re-docked in the active site of *E. coli* DNA gyrase B kinase (PDB code: 1KZN) [28, 29] which revealed a score energy of -6.48 kcal/mol (**Table 1** and **Figure 1**). As shown in **Figure 1-5** and **Table 1**, some compounds (3a, 3d, 3e, and 3f) can create a strong hydrogen bond with Asn46, Asp73, and Thr165 at a distance of 3.91-6.31Å, which is consistent with the decomposition analysis of the electrostatic interaction.

Table 1: Energy-based interactions for *N*,2-diphenylquinazolin-4-amine derivatives docked into DNA gyrase.

Compound	Estimated free energy of binding (kcal/mol)	Hydrogen bond (Å)
3a	-4.75	Asn 46 (4.43), Arg 136 (6.31)
3b	-5.92	-
3c	-6.13	-
3d	-3.05	Asn 46 (4.16)
3e	-4.21	Asp 73 (4.39)
3f	-3.69	-
3g	-4.24	Asn 46 (4.56),
chlorobiocin	-6.48	Asp 73 (4.04), Asn 46 (4.46), Thr 165 (3.91)

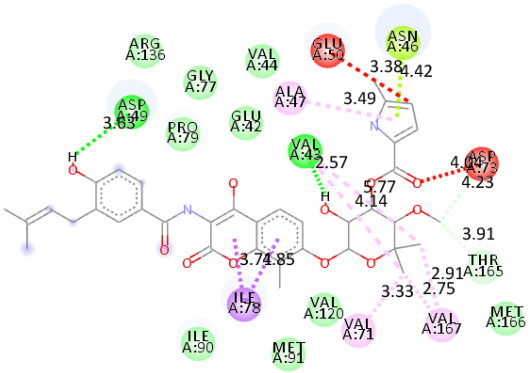


Figure 1. Diagram illustrating the 2D binding patterns of chlorobiocin onto the ATP-active pocket of *E*. coli DNA gyrase B kinase (PDB code: 1KZN).

The *N*-(4-nitrophenyl)-2-phenylquinazolin-4-amine derivative 3c, which displayed the most potent inhibitory activity, gave the highest binding energy (-6.13 kcal/mol). Moreover, the *N*-(3-nitrophenyl)-2-phenylquinazolin-4-amine derivative 3b, showed the second-best docking score, with a binding energy of -5.92 kcal/mol. But, no hydrogen bonds interaction for 3c, and 3b was detected (**Table 1** and **Figure 2**).

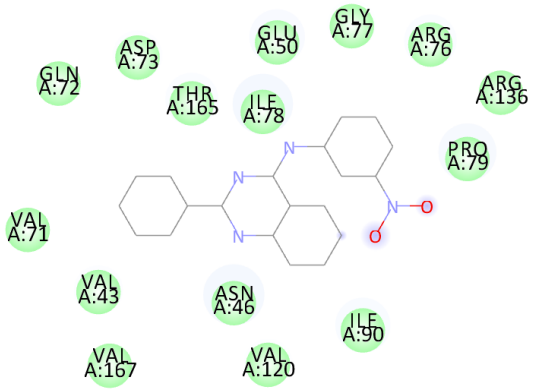


Figure 2. Diagram illustrating the 2D binding patterns of compound 3b onto the ATP-active pocket of *E*. coli DNA gyrase B kinase (PDB code: 1KZN).

Regarding the binding of 3a and 3d with *E. coli* DNA gyrase B, there was an H-bond interaction between the NH proton of *N*-(4-methoxyphenyl)-2-phenylquinazolin-4-amine

with the side chain of Asn46 (distance: 4.43 Å), also exhibited H-bond interaction was found between OCH_3 group with the side chain of Arg136 for 3a (**Table 1** and **Figure 3**).

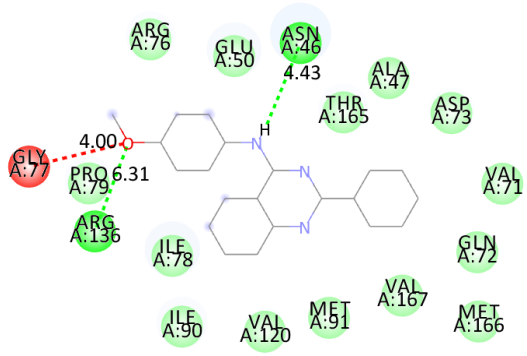


Figure 3. Diagram illustrating the 2D binding patterns of compound 3a onto the ATP-active pocket of *E*. coli DNA gyrase B kinase (PDB code: 1KZN).

Furthermore, the NH_1 of quinazoline moiety of 3e formed with the side chain of Asp73 a favorable hydrogen bonding (distance: 4.39 Å) (**Table 1** and **Figure 4**), and also there was an H-bond interaction between the NH proton of phenyl hydrazine moiety with the side chain of Asn46 for 3g (**Table 1** and **Figure 5**).

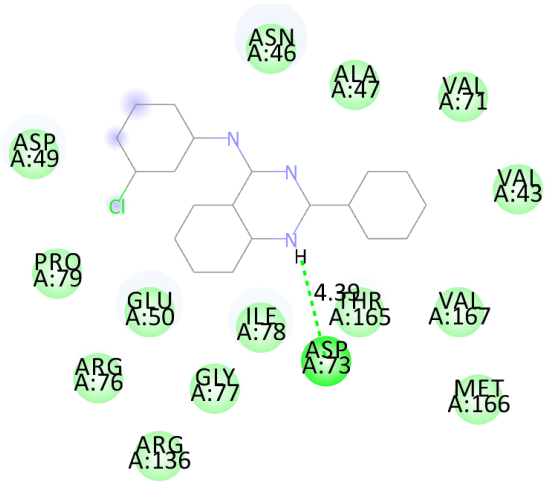


Figure 4. Diagram illustrating the 2D binding patterns of compound **3e** onto the ATP-active pocket of *E*. coli DNA gyrase B kinase (PDB code: 1KZN).

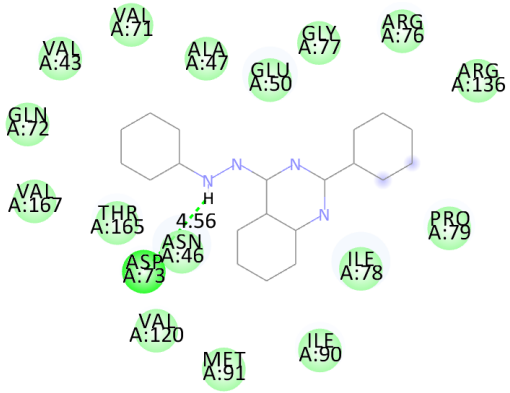


Figure 5. Diagram illustrating the 2D binding patterns of compound 3g onto the ATP-active pocket of *E*. coli DNA gyrase B kinase (PDB code: 1KZN).

3.3. Antibacterial Activity

In the biological assay, the activity of the target compounds against the Gram-positive strains was more potent than their activity against the Gram-negative strains. In turn, *N*-(3-chlorophenyl)-2-phenylquinazolin-4-amine 3e showed potent activity against *S. aureus* (MIC = 0.0039 mg/mL), equal to that of the reference drug. The 1-phenyl-2-(2-phenylquinazolin-4-yl) hydrazine 3g also revealed potent activity against *P. aeruginosa* at 0.0625 mg/mL concentration. It seems that lipophilicity could improve the antibacterial activity of the newly synthesized *N*,2-diphenylquinazolin-4-amine derivatives (3a-g) (**Table 2**) [30, 31]. Structure-activity relationship analysis based on the observed results demonstrated that in compounds 3e and 3f, the presence of electron-withdrawing groups (chlorine group) at the meta and para positions of the phenyl ring could be responsible for good activities because of its inductive effect. Results of the antifungal study showed that almost all of the tested compounds have remarkable antifungal activity against *C. albicans*, significantly with compounds 3c and 3g having the highest activities at 0.0625 mg/mL concentration (**Table 3**).

Table 2: MIC (mg/mL) results of synthesized compounds against various bacteria.

Code	*E. coli*		*P. aeruginosa*		*S. aureus*		*B. subtilis*		*L. rhamnosus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	0.5	>2	0.25	> 2	0.5	2	0.5	> 2	1	2
3b	0.5	1	0.25	2	0.015	0.5	0.5	2	1	1
3c	0.125	0.5	0.125	0.25	0.25	> 0.5	0.125	> 1	0.25	0.5
3d	0.5	> 2	0.125	1	0.5	2	0.5	>2	1	2
3e	0.5	2	0.25	0.5	0.003	0.031	0.5	1	1	1
3f	2	>2	0.25	2	0.007	0.015	0.5	> 2	1	1
3g	0.125	0.25	0.062	0.25	0.5	0.125	0.125	0.125	0.25	0.25
Cipro	0.000	0.5	0.001	0.002	0.003	> 0.003	0.5	0.5	-	-

Table 3: MIC (mg/mL) results of synthesized compounds against *C. albicans*.

Code	3a	3b	3c	3d	3e	3f	3g	Nystatin
MIC	0.25	0.25	0.0625	0.25	0.25	0.25	0.0625	> 1
MBC	> 2	> 2	> 1	> 2	> 2	> 2	> 1	> 1

4. Conclusion

In this study, synthesis, molecular docking, and evaluation of the antimicrobial activity of seven novel *N*,2-diphenylquinazolin-4-amine derivatives were reported. All compounds showed moderate to good antibacterial activity, while remarkable antifungal activities were observed for these compounds. Computational studies were performed by automated docking of ligands to the binding sites of DNA gyrase. The results revealed that compound 3c showed minimum binding energy (-6.13 kJ/mol) and so, indicated a strong binding affinity towards DNA gyrase. Further developments are in progress to optimize new *N*,2-diphenylquinazolin-4-amine derivatives as potential antibiotic drug candidates in the future.

Ethical Issues

This study was approved by the Ethics Committee of Lorestan University of Medical Sciences (code: IR. LUMS. REC. 1398.187. and IR. LUMS. REC 1399.369).

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Conflict of interest

The authors declare to have no conflict of interest.

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