Synthesis, antimicrobial evaluation and docking studies of some novel quinazolinone Schiff base derivatives

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Abstract

The quinazolin-4(3H)-one structural motif possesses a wide spectrum of biological activities. DNA gyrase play an important role in induction of bacterial death. It has been shown that many quinazolin-4(3H)-one derivatives have antibacterial effects through inhibition of DNA gyrase. Based on this information we decided to synthesize novel quinazolinone Schiff base derivatives in order to evaluate their antibacterial effects. A series of novel quinazolinone Schiff base derivatives were designed and synthesized from benzoic acid. The potential DNA gyrase inhibitory activity of these compounds was investigated using *in silico* molecular docking simulation. All new synthesized derivatives were screened for their antimicrobial activities against three species of Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella entritidis* and three species of Gram-positive bacteria comprising of *Staphylococcus aurous*, *Bacillus subtilis*, *Listeria monocitogenes* as well as for antifungal activities against *Candida albicans* using the conventional micro dilution method. Most of the compounds have shown good antibacterial activities, especially against *E. coli* at 128 μg/mL concentration while no remarkable antifungal activities were observed for these compounds. All the synthesized compounds exhibit dock score values between -5.96 and -8.58 kcal/mol. The highest dock score among them was -8.58 kcal/mol for compound **4c**.

Keywords: Schiff base; Synthesis; Quinazoline-4(3H)-ones; Antibacterial activity; Docking study

INTRODUCTION

Due to multiple resistances to antibiotics, many researchers around the world have focused their efforts on the development of new antimicrobial agents (1-3). DNA gyrase is an enzyme within the class of topoisomerase that catalyze changes in the topology of DNA. The presence of DNA gyrase in all bacteria except higher eukaryotes, makes it a drug target for antibacterial studies (4,5). Over the past decade, the quinazolin-4(3H)-one structural motifs containing azomethine (-C=N) functional group has become one of the main areas of interest due to possessing a wide spectrum of biological activities including antibacterial, antifungal, anticonvulsant, anti-inflammatory, antiviral, antihyperlipidemic, anti-tubercular, CNS depressant, anti-tumor, analgesic, antimalarial, and anti-cancer effects (6,7). Azomethine functional groups are formed when a primary amine reacts with a carbonyl compound (8,9). Quinazolinone as an important pharmacophore for a range of inhibitors such as tyrosine kinase, thymidylate synthase and 5HT7 receptor could be grafted by azomethine functional group to exhibit better pharmacological properties. Gefitinib (ZD-1839, Iressa_) and erlotinib (OSI-774, Tarceva), two quinazoline based structures, are already in clinic as important epidermal growth factor receptor inhibitors (Scheme 1) (10-12).

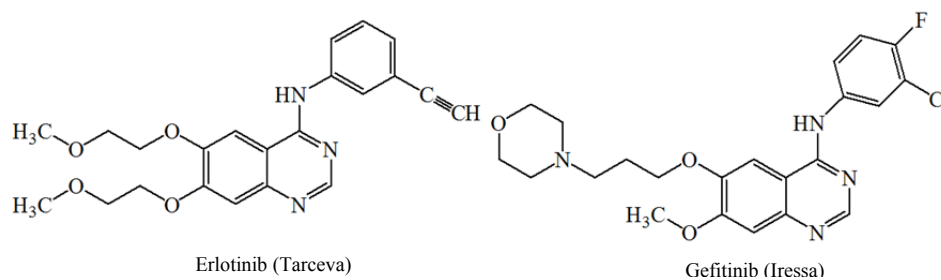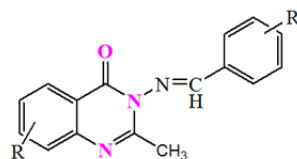| Access this article online	
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Scheme 1. Chemical structure of some epidermal growth factor receptor tyrosine kinase inhibitors.

Entry	R	R'
a	H	H
b	H	2-NO$_2$
c	H	4-NO$_2$
d	H	2-OH,5-Br
e	H	4-Cl
f	6-Cl	H
g	6-Cl	2-NO$_2$
h	6-Cl	4-NO$_2$
i	6,8-diCl	H
j	6,8-diCl	2-NO$_2$
k	6,8-diCl	4-NO$_2$
l	6,8-diCl	2-OH,5-Br
m	6,8-diCl	4-Cl

Scheme 2. Schematic representation of final compounds 4a-m.

The molecular docking technique is one of the most interesting tools applied in pharmaceutical research. In this method one can dock small molecules into receptor targets which helps to predict ligand conformation and orientation within a targeted binding site (2,3). Moreover, to the best of our knowledge, no docking study of 3-(arylidene-amino)-2-methyl quinazolin-4(3H)-one with DNA-gyrase has been reported so far.

In the present work we have synthesized some new derivatives of 3-(arylidene-amino)-2-methyl quinazolin-4(3H)-one containing chlorine atoms at positions 6 and 8, methyl group at position 2 and various aromatic aldehydes at position 3 (Scheme 2).

In vitro antimicrobial activities of these compounds were evaluated against both Gram-positive and Gram-negative bacteria as well as fungal strains. The synthesized compounds were docked into the binding sites of DNA gyrase and their binding energies were calculated.

MATERIALS AND METHODS

All the chemicals were of synthetic grade and solvents used in this study were purchased from Merck Co. (Merck, Germany). The reactions were monitored by thin-layer chromatography on silica gel (F245 Merck plates, Merk, Germany). Melting points were recorded in the open capillaries using electrothermal 9200 melting point apparatus and uncorrected. ^{1}H NMR spectra were obtained on a Bruker 400 MHz spectrometer (United States) in deuterated dimethyl sulfoxide (DMSO-d6) using tetramethylsilane as an internal reference. Mass spectra were measured on a Shimadzu mass spectrometer (Japan). The infrared radiation spectra were determined on a WQF-510 Fourier-transform infrared (FT-IR) spectrophotometer (BRAIC Co., China) using the KBr disk method. For *in silico* protein-ligand docking simulation, AutoDock 4.2, Discovery studio 2.5, Hyper Chem 7.0, and Lig Plot software packages

were used. Microorganism in antibacterial test was purchased from the Persian Type Culture Collection (Iran). Muller Hinton agar and Sabouraud dextrose agar were purchased from Merck (Germany). Standard antifungal drug (ketoconazole) and antibacterial drug (ciprofloxacin) (Farabi, Iran) were used for comparison.

Molecular docking studies

The novel quinazolinone Schiff base derivatives were subjected to dock in the active site of DNA gyrase enzyme using Autodock 4 software. We investigated the theoretical binding mode of 13 ligands at the chlorobiocin binding site using molecular docking modeling. Molecular docking studies were performed for these ligands to understand the ligand-receptor possible intermolecular interactions in detail. Chlorobiocin is an amino coumarin antibiotics that act by an inhibition of the DNA gyrase enzyme involved in the cell division of bacteria (13-16).

The crystal structure of the DNA gyrase (PDB code 1KZN) with resolution 2.3 Å was chosen as the protein model for the present study (17). Water molecules and ligand were removed from the protein file. The resulting crystallography structure was imported in AutoDock. The binding features of 13 synthesized compounds with DNA gyrase were evaluated in the same manner of binding of chlorobiocin as a well-known enzyme inhibitor (18).

The structures of the ligands were optimized using HyperChem 7.0 software (version 7.0; Hypercube, Inc., Gainesville, FL, USA; http://www.hyper.com). Using the MM+ molecular mechanical force field, 3D geometry optimization calculations for each ligand were performed. The ultimate conformations were calculated with the semi empirical parameterized model number 3 (PM3) method. The molecular structures were optimized using the Polak-Ribiere algorithm until the root mean square gradient was 0.01 kcal/mol/Å. Geometry optimization was run many times with different starting points of each 13 ligand (19).

Docking was performed using the routine procedure and default parameters of molecular docking AutoDock 4.2 software and implemented empirical free energy function (19). Only polar hydrogens were added to the protein and all water molecules were removed from the protein file in AutoDock Tools. In the docking protocol, ligands were assumed to be flexible molecules and the docking software was allowed to rotate all rotatable bonds of the ligands to obtain the best and optimized conformer of the ligands within the active site of the enzyme. The native ligand, chlorobiocin, was redocked to the binding site. The grid box was centered with the coordinates x = 19.259, y = 29.159, z = 42.461 for DNA gyrase (PDB code 1KZN). Grid box dimensions were 46 × 46 × 46 with a 0.375 Å grid points spacing. Grid maps were calculated by Autogrid4. A lamarckian genetic algorithm program with an adaptive whole method search in the Autodock was used to calculate the different ligand conformers (20.21). At the end of docking experiment with 200 runs, a cluster analysis was performed. Conformations were clustered according to the root mean square deviation tolerance of 2.0 Å and were ranked according to the binding free energy (22). Among the various conformations of these ligands obtained from the docking procedure, the conformation with the best scored pose and with the lowest binding energy was selected for these ligands (19,20). LigPlot software was used to investigate the hydrophobic and hydrogen bonding interactions between the ligand and the enzyme.

Chemistry

Quinazolinone Schiff base derivatives were prepared according to the main procedure introduced by Rezvan Rezaee Nasab, *et al.* (23).

Antimicrobial activity

The antimicrobial activities of the synthesized compounds were studied using broth micro dilution method against three Gram-positive bacteria of *Staphylococcus aureus* PTCC 1337, *Bacillus subtilis* PTCC 1023, and *Listeria monocitogenes* PTCC 1165 and three Gram-negative bacteria including *Escherichia coli* PTCC 1338, *Pseudomonas*

aeruginosa PTCC 1074, and *Salmonella entritidis* PTCC 1091 and antifungal activity was screened against *Candida albicans* PTCC 5027 obtained from Persian Type Culture Collection. Using the micro plate alamar blue assay, minimum inhibitory concentration (MIC) was determined (24,25).

In this test dimethyl sulfoxide (DMSO) was used as a solvent for stock solution and diluted with water. Therefore, the final concentration of DMSO was no greater than 1%. A single 96-well micro dilution plate was used. Muller-Hinton broth and RPMI 1640 were used as media for bacterial and fungal growth, respectively (26,27).

RESULTS

Table 1. summarizes the binding characterizations between synthesized compounds and DNA gyrase. According to the data presented in this table ligands interact with the DNA-gyrase binding site through hydrogen bonding. Amongst various conformations of the ligands obtained from the docking procedure the conformation with the best scored pose, with the lowest binding energy (~ -6 – -9 kcal/mol), and as the most populated cluster was selected. However, the best scored pose for **4d** and **4l** (-6.84 and -6.67

kcal/mol, respectively) was not the most populated cluster. The next cluster (-5.96 and -6.65 kcal/mol) had a similar position and orientation with the reference structure and at the same time was the most populated cluster (number of neighbors = 143 and 118, respectively).

Figures 1-4 show 3D schematic presentations of compounds **4a-4l** as well as chlorobiocin while docked into the chlorobiocin binding site and supports the idea that the compounds are well incorporated into the binding pocket. Dock pose of each ligand was analyzed for interactions with the receptor. These hydrophobic sites of the ligands are conserved in the majority of the structures (Figs. 1-4). The residue that interacts through a hydrogen bonding with the acceptor/donor site of the ligand differs depending on the ligand. The binding patterns of different ligands are also slightly different.

In this study, the results have shown that synthesized novel quinazolinone Schiff base derivatives followed Lipinski's RO5 (data not shown) (28).

The designed compounds were prepared through conventional synthetic pathways and characterized by spectroscopic methods. Structural properties of the newly synthesized compounds are shown below.

Table 1. Energy-based interactions and hydrogen bonds for 13 novel quinazolinone Schiff base derivatives and chlorobiocin docked into DNA gyrase.

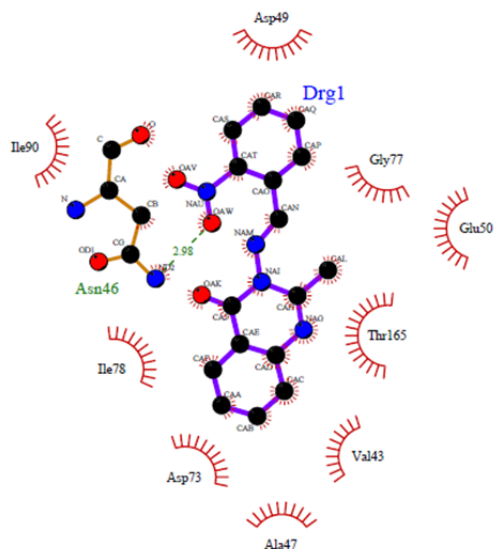| NO	Estimated free energy of binding (kcal/mol)	Hydrogen bond
4a	-7.44	Asp46* (3.17 Å)
4b	-6.48	Asp46* (2.98 Å)
4c	-8.58	-
4d	-5.96	Asp46* (2.89 Å)
4e	-7.69	Asp46* (3.33 Å)
4f	-6.57	-
4g	-7.18	Thr165 (2.81 Å)
4h	-7.10	-
4i	-6.79	-
4j	-6.13	-
4k	-6.77	Arg136* (2.56 Å)
4l	-6.65	Thr165 (3.10 Å), Arg76 (2.90 Å)
4m	-7.06	-
chlorobiocin	-6.48	Asp73 (2.54 Å), Arg136 (2.51 Å)

Fig. 1. Docked conformation of compound **4b** in the binding site of DNA-gyrase. Hydrogen bonds are shown by green dashed line.
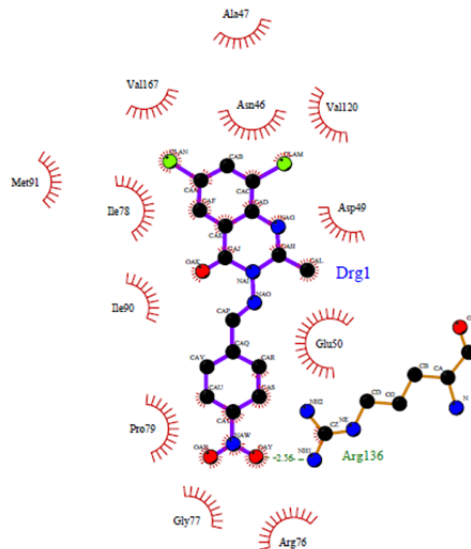

Fig. 2. Docked conformation of ligand structure **4k** in the binding site of DNA-gyrase. Hydrogen bonds are shown by green dashed line.
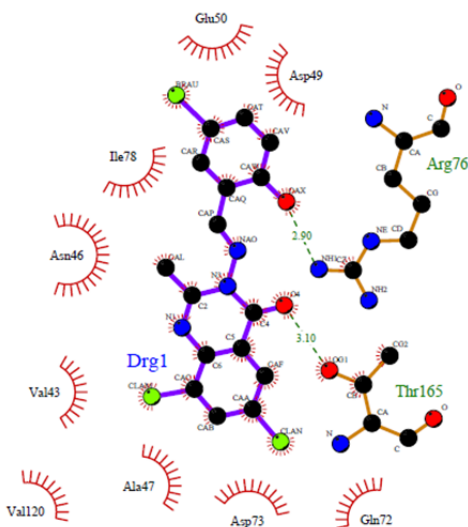

Fig. 3. Docked conformation of ligand structure **4l** in the binding site of DNA-gyrase. Hydrogen bonds are shown by green dashed line.
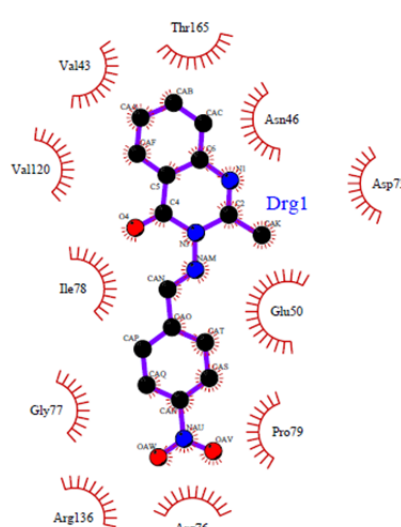

Fig. 4. Binding model of compound **4c** for the best docked pose in the DNA-gyrase active site.

3-(2-nitrobenzylideneamino)-2-methyl-quinazolin-4(3H)-one (4b)

Light yellow crystals, yield: 76%. m.p: 245-246 °C, (MS: m/z (%): 308 (M+, 100), $C_{16}H_{12}N_4O_3$ M.W. 308), FT-IR (KBr cm^{-1}): 3064.33 (C-H arom, str.), 2962.13, 2925.48 (C-H aliph, str.), 1667.16 (N-C=O, str.), 1610.27 (C=N, str.).^{1}H NMR (400 MHz, DMSO-d$_6$) δ in ppm: 9.4 (1H, s, -N=CH-), 8.2 (1H, t, j = 4 Hz, Ar-H), 8.19 (1H, d, j = 3.2 Hz, Ar-H), 8.16 (1H, s, Qu-H), 7.9 (1H, t, j = 7.6 Hz, Ar-H), 7.86 (1H, t, j = 7.6 Hz, Ar-H), 7.84

(1H, t, j = 8 Hz, Qu-H), 7.6 (1H, d, j = 7.2 Hz, Qu-H), 7.5 (1H, t, j.= 8 Hz, Qu-H), 2.5 (3H, s, CH$_3$).

3-(5-bromo-2-hydroxybenzylideneamino)- 2 - methylquinazolin-4(3H)-one (4d)

White crystals yield: 70%. m.p: 235-236 °C, (MS: m/z (%): 357 (M+, 100), $C_{16}H_{12}BrN_3O_2$ M.W. 357), FT-IR (KBr cm^{-1}): 3398.92 (OH, str.), 3066.26 (C-H arom, str.), 2924.52 (C-H aliph, str.), 1683.55 (N-C=O, str.), 1605.45 (C=N, str.). ^{1}H NMR (400 MHz,

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DMSO-d$_6$) δ in ppm:10.911 (1H, broad, OH,), 9.1 (1H, s, -N=C<u>H</u>-), 8.2 (1H, d, j = 8 Hz, Qu-H), 8.1 (1H, s, Ar-H), 7.9 (1H, t, j = 8 Hz, Qu-H), 7.7 (1H, d, j = 8 Hz, Ar-H), 7.5(1H, t, j = 8 Hz, Qu-H), 7.0 (1H, d, j = 8.8 Hz, Qu-H), 2.6 (3H, s, CH$_3$).

3-(4-chlorobenzylideneamino)-2-methyl-quinazolin-4(3H)-one (4e)

Pale yellow crystals, yield: 90%. m.p: 203-231 °C, (MS: m/z (%): 297 (M+, 100), 299 (M+2) C$_{16}$H$_{12}$ClN$_3$OM.W. 297.07), FT-IR (KBr cm^{-1}): 3087.48 (C-H arom, str.), 2928.38 (C-H aliph, str.), 1679.69 (N-C=O, str.), 1598.7 (C=N, str.). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ in ppm: 9.0 (1H, s, -N=C<u>H</u>-), 8.1 (1H, d, j = 8 Hz, Qu-H), 8.0 (2H, d, j = 7.2 Hz, Ar-H), 7.8 (1H, t, j = 8 Hz, Qu-H), 7.6 (2H, d, j = 8 Hz, Ar-H), 7.5(1H, t, j = 8 Hz, Qu-H), 2.5(3H, s, CH$_3$).

3-(benzylideneamino)-6, 8-dichloro-2-methyl-quinazolin-4(3H)-one (4i)

Pale yellow crystals, yield: 89%. M.p: 211-212 °C, (MS: m/z(%): 331 (M+, 100), 333 (M+2), 335 (M+4) C$_{16}$H$_{11}$Cl$_2$N$_3$O M.W. 331), FT-IR (KBr cm^{-1}): 2379.73 (C-H arom, str.), 2358.52, 2348.87 (C-H aliph, str.), 1672.95 (N-C=O, str.), 1595.81 (C=N, str.). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ in ppm: 8.9 (1H, s, -N=C<u>H</u>-), 8.1 (1H, d, j = 2 Hz, Qu-H), 8.0 (1H, d, j = 2 Hz, Qu-H), 7.9 (1H, d, j = 6.8 Hz, Ar-H), 7.6 (1H, t, j = 6.8 Hz, Qu-H), 7.5 (1H, t, j = 6.8 Hz, Ar-H), 2.5 (3H, s, CH$_3$).

3-(5-bromo-2-hydroxybenzylideneamino)-6, 8-dichloro-2-methylquinazolin-4(3H)-one (4l)

Yellow solid, yield: 80%. M.p: 220-222 °C, (MS: m/z (%): 424 (M+, 100), 426 (M+2), 428 (M+4) C$_{16}$H$_{10}$BrCl$_2$N$_3$O$_2$M.W. 424), FT-IR (KBr cm^{-1}): 3477.03 (C-H arom, str.), 3416.28 (OH, str.), 3071.08, 2924.52 (C-H aliph, str.), 1683.55 (N-C=O, str.), 1607.38 (C=N, str.). ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ in ppm: 10.9 (1H, broad, OH), 9.1 (1H, s, -N=C<u>H</u>-), 8.19 (1H, d, j = 2.4Hz, Qu-H), 8.14 (1H, d, j = 2.4Hz, Qu-H), 8.09 (1H, d, j = 2.8Hz, Ar-H), 7.6 (1H, d, j = 8.8Hz, Ar-H), 7.0 (1H, d, j = 8.8 Hz, Ar-H), 2.6 (3H, s, CH$_3$).

3-(4-chlorobenzylideneamino)-6, 8-dichloro-2-methylquinazolin-4(3H)-one (4m)

Light yellow crystals, yield: 90%. m.p: 210-211 °C, (MS: m/z (%): 364 (M+, 100), 366 (M+2), 368 (M+4) C$_{16}$H$_{10}$Cl$_3$N$_3$OM.W. 364), FT-IR (KBr cm^{-1}): 3219.7 (C-H arom, str.), 3001.1, 2900.2 (C-H aliph, str.), 1679.69 (N-C=O, str.), 1598.7 (C=N, str.). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ in ppm: 8.9 (1H, s, -N=C<u>H</u>-), 8.1 (1H, d, j = 2.4 Hz, Qu-H), 8.0 (1H, d, j = 2.4 Hz, Qu-H), 7.9 (2H, d, j = 8.4 Hz, Ar-H), 7.6 (2H, d, j = 8.4 Hz, Ar-H), 2.6 (3H,s, CH$_3$).

All the synthesized compounds were tested for antibacterial activities against three species of Gram-negative bacteria and three species of Gram-positive and also for antifungal activities. The results of biological effects are shown in Table 2 and 3.

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

| Code | Gram-negative bacteria | | | | | | Gram-positive bacteria | | | | | |
| | Escherichia coli | | Pseudomonas aeruginosa | | Salmonella entritidis | | Staphylococcus aureus | | Bacillus subtilis | | Listeria monocitogens | |
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4a	512	G	512	G	512	G	64	256	512	G	512	G
4b	512	G	512	G	512	G	512	G	512	G	512	G
4c	512	G	512	G	512	G	32	64	256	512	512	G
4d	512	G	512	G	512	G	512	G	512	G	512	G
4e	128	256	32	64	512	G	32	64	128	256	512	G
4f	512	G	256	512	256	512	128	256	256	512	256	G
4g	G	G	256	G	256	512	64	128	256	512	256	512
4h	256	512	256	512	512	G	128	256	256	G	256	G
4i	256	G	256	512	512	256	256	512	256	512	256	512
4j	256	512	256	G	256	512	256	G	32	64	256	G
4k	G	G	256	G	256	512	256	512	256	512	256	512
4l	256	512	256	512	256	G	256	512	256	512	256	G
4m	128	256	128	256	128	256	128	256	128	256	128	256

(G), growth; (MIC), minimum inhibitory concentration; (MBC), minimum bactericidal concentration; (NA), not applicable. Ciprofloxacin used as standard antibacterial agent.

Table 3. MIC (μg/mL) and results of synthesized compounds against *Candida albicans*

	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m
MIC	512	512	512	512	512	256	256	256	256	256	256	512	128
MBC	G	G	G	G	G	512	512	512	512	G	G	G	256

(MIC), minimum inhibitory concentration; (MBC), minimum bactericidal concentration.

DISCUSSION

As shown in Fig. 1, some compounds (**4b**, **4d**, **4e,** and **4h**) can create a strong hydrogen bond with Asn46 at distance 2.89 Å - 3.33 Å, which is consistent with the decomposition analysis of the electrostatic interaction. It is interesting that more complex stabilization might result from the hydrogen bonds between these ligands and Arg136 via nitro electron withdrawing substitution on the phenyl ring (for **4h** and **4k**) (Fig. 2). Although these interactions were also observed for these derivatives, these are different from those present in **4g** (Thr165) and **4l** (Thr165 and Arg76) (Fig. 3) which may be responsible for the activity variations. These results are consistent with the X-ray cocrystal structure and previous studies indicating the important roles of those residues (29-32). No hydrogen bonds interaction for **4a**, **4c**, **4f**, **4i**, **4j**, **4l,** and **4m** was predicted (Fig. 4).

These compounds also significantly stabilize the DNA gyrase through hydrophobic contacts with Ala47, Glu50, Val71, Asp73, Arg76, Gly77, Ile78, Pro79, Met91, Val120, Thr163, Thr165, and Val167. Compounds were deeply embedded into the hydrophobic pocket. In all compounds it is clear that the hydrophobic pocket of the inhibitor binding site was occupied by quinazolinone or phenyl along with the groups substituted on these rings.

The docking protocol adopted in this investigation was validated by docking of chlorobiocin as a well-known inhibitor to the energy minimized DNA gyrase protein. The residues Asp73, Asn46, and Arg136 are important in making hydrogen bond (33-35). Most of the compounds in this study also showed a strong hydrogen bond with Asn46.

The highest dock score in docking protocol for these series was -8.58 kcal/mol for compound **4c**. Rest of the molecules showed a proper dock scores ranging from -5.96 to -8.58 kcal/mol. Thus, the binding model reported here, suggests that these quinazolinone Schiff base derivatives behave as DNA gyrase inhibitors and show some key structural points to be considered in future optimization.

In the biological assay, most of the synthesized compounds showed potent activities against both the Gram-positive and Gram-negative bacterial species. Obtained results of screened compounds against Gram negative organisms demonstrated that compounds **4e** and **4m** had the highest activities against *E.coli* at 128 μg/mL. Compound **4e** has shown the best activity against *P. aeruginosa* and *S. aureus* at 32 μg/mL. Compound **4c** has shown the highest activity at 32 μg/mL against *Staphylococcus aureus* strain.

Structure-activity relationship studies based on the observed results showed that in **4a-4l**, presence of electron-withdrawing groups (chlorine group) at the para positions of the phenyl ring could be responsible for good activities because of its inductive effect. Interestingly, compounds that are substituted with 6, 8-dichloro have shown better activity than 6-chloro substituted compounds. Furthermore, these compounds have better activity than unsubstituted quinazolinone derivatives (36,37). Results of antifungal study showed that almost all of the tested compounds have no antifungal activity against *C. albicans*, except for compound **4m** which showed good activity at 128 μg/mL (Table 3).

CONCLUSION

All of the synthesized compounds showed good antibacterial activity, especially against *E.coli* at 128 μg/mL concentration while no remarkable antifungal activities were observed for these compounds. In the docking experiments, all compounds were able to interact in a manner similar to the known inhibitors with the residues located in the DNA-gyrase binding site. All derivatives exhibited the docking ΔG binding values

lower than chlorobiocin except for **4d**. The highest dock score in docking protocol for these series was -8.58 kcal/mol for compound **4c**. This study indicates that quinazolinone Schiff base derivatives could be a suitable scaffold for DNA gyrase inhibitors and may be helpful in the search for novel classes of potent antibiotic agents.

ACKNOWLEDGEMENTS

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