Biological Evaluation and Molecular Docking of Substituted Quinazolinones as Antimicrobial Agents

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Abstract: Antibiotic resistance in the community is a growing public health concern due to the continued emergence of multi drug resistant bacterial strains. In view of this fact, the design and synthesis of newer antibacterials are of immense significance and continue to attract the attention of numerous medicinal chemists. The aim of this study was to investigate the effect of four quinazolinone derivatives (Ia, II, III and IV) on the microbial cell morphology and genes coded for rRNA subunits. It was extended also to elucidate the effect of the most active derivatives on DNA-gyrase enzyme by performing a molecular docking study. Quinazolinone derivatives revealed good anti-bacterial activities, especially against Gram-positive strains through their interaction with cell wall and DNA structures. The tested compounds showed moderate activity against fungal strains through affecting the internal structures of fungal cell in addition to studied genes. Compound II was found to be the most active gyrase inhibitor as illustrated by the docking study. Conclusion: the type and position of chemical substituents confer the antimicrobial activity of quinazolinone.

Key words: Quinazolinone, Antimicrobial activity, DNA melting temperature, Gyrase inhibitor.

INTRODUCTION

During the past decades, the human population affected with life threatening infectious diseases caused by multidrug resistant Gram-positive and Gram-negative pathogen bacteria increased to an alarming level around the world. Due to this reason, it is imperative to design and develop new antibacterial or antifungal agents with novel chemical structure preferably having different modes of action rather than analogues of the existing ones (Tale et al., 2011). Quiazolinones and their derivatives constitute an important class of heterocyclic compounds and occupy an important position in medicinal chemistry (Aanandhi et al., 2011). As medicines, many of these derivatives display anti-inflammatory, anti-tubercular, antihistaminic, anti-diabetic, antidiuretic, antihypertensive, phosphodiesterase inhibition and anticancer agents (Vijaychand et al., 2011). Several reports have demonstrated the effect of quinazolinone derivatives on bacteria and fungi and revealed that most of these compounds exhibited anti-bacterial and anti-fungal activities (Patel et al., 2006, Ouyang et al., 2006, Boyapati et al., 2010).

The 4-quinolones such as ciprofloxacin and ofloxacin are established synthetic antibacterial agents as DNA gyrase inhibitors (Boehm et al., 2000). Substituted quinazolines were reported as isosteres of quinolones to show a variety of antibacterial activities with DNA gyrase inhibition (Huband et al., 2007, Tran et al., 2007)]. DNA gyrase, consisting of the subunits GyrA and GyrB, is a member of the type II family of topoisomerases that control the topological state of DNA in cells (Wang, 2009). DNA gyrase couples ATP hydrolysis by the GyrB subunit to supercoiling of DNA, which is required for maintenance of DNA topology during the replication process. It is an essential enzyme across bacterial species, and inhibition results in disruption of DNA synthesis and, subsequently, cell death (Eakin et al., 2012).

In view of this fact, the design and synthesis of newer anti-bacterials and anti-fungals are of immense significance and continue to attract the attention of numerous medicinal chemists. Consequently, the aim of the present study was to assess the effects of four synthesized quinazolinone derivatives on some pathogenic microbial strains through their effects on cell morphology, genes coded for rRNA subunits and bacterial gyrase.

MATERIALS AND METHODS
All chemicals and solvents were of high analytical grade. Chemicals and primers were purchased from Sigma Chemical Company.

**Synthesis of substituted quinazolinone derivatives (Scheme 1, Table I):**

All melting points are not corrected. The infrared (IR) spectra were recorded on a pye Unicomp SP3-200 spectrophotometer using KBr disks optics.

**Synthesis of 2-(6-bromo-4-oxo-3,4-dihydroquinazolin-2-yl) acetonitrile (compound Ia) and 2-amino quinazolin-4(3H) one (compound Ib):**

5-Bromo anthranilic acid or anthranilic acid (0.01 mol) and cyanoacetamide or thiourea (0.01 mol) were taken up in 30 ml butanol and few drops of triethylamine and the mixture was heated under reflux for a period of 6 hours. After that, the reaction mixture was added to ice-cold water and the solid product was collected by filtration and recrystallised from the proper solvent to give yellowish brown (Ia) or brown (Ib) powders [for Ia: m.p. 250-252°C and yield 70%. For Ib: m.p. 170°C and yield 70%].

**Synthesis of ethyl 2-(6-bromo-2-cyanomethyl)-4-oxo-quinazolin-3(4H)-yl acetate (compound II):**

Compound Ia (0.01 mol) and ethyl chloroacetate (0.02 mol) were taken up in 30 ml pyridine and the mixture was heated under reflux for a period of 3 hours. The solid was precipitated by cooling in a mixture of ice and dilute hydrochloric acid, filtered off, washed well with water and recrystallised from the proper solvent to give chocolate powder [m.p. 218°C and yield 65%].

**Synthesis Of Arylidine Derivatives (compound III):**

To (0.01 mol) of Ib in 30 ml of absolute ethanol and (0.05 mol) of sodium ethoxide, benzoyl acetone (0.01 mol) was added with strong stirring for 2 hours. The precipitate was filtered and recrystallised from the proper solvent to give white crystals [m.p. 200°C and yield 65%].

**Synthesis of 2-(6-bromo-4-oxo-3,4-dihydroquinazolin-2-yl) acetamide (compound IV):**

Compound Ia (0.01 mol) and 40% sulphoric acid (20 ml) were heated on a water bath under reflux for a period of 2 hours. The solid that precipitated after cooling was collected and recrystallized from the proper solvent to give brown powder [m.p. 210°C and yield 75%].

**Antibacterial Activity:**

All the compounds were screened for their in vitro antibacterial and antifungal activities. The specified stains of organisms were procured from the Regional Center for Mycology and Biotechnology, Al-Azher University, Cairo, Egypt. Strains and cultivation conditions of the tested microorganisms are shown in table (2).

The antimicrobial activities were evaluated in vitro by agar diffusion method and minimum inhibition concentration (MIC) was performed by micro titre broth dilution method (Smania et al., 1999).
Table 1: Spectral data of the synthesized quinazolinone derivatives.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Molecular formula / Molecular weight</th>
<th>IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>C8H6N3OBr / 264</td>
<td>-3407.6 cm⁻¹ (N-H or OH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1684.4 cm⁻¹ (C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-2269 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1622.7 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-539.4 cm⁻¹ (C-Br)</td>
</tr>
<tr>
<td>Ib</td>
<td>C8H7N3O / 161</td>
<td>-3359-3275 cm⁻¹ (NH₂)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3275 cm⁻¹ (N-H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1710 cm⁻¹ (C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1612 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td>II</td>
<td>C14H12N3O3Br / 350</td>
<td>-2200 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1676 cm⁻¹ (C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1590 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-590 cm⁻¹ (C-Br)</td>
</tr>
<tr>
<td>III</td>
<td>C18H15N3O2Br / 305</td>
<td>-3380 cm⁻¹ (N-H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1650 cm⁻¹ (C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1597 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td>IV</td>
<td>C10H8N3O2Br / 282</td>
<td>-3468-3358 cm⁻¹ (NH₂)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1676 cm⁻¹ (C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1590 cm⁻¹ (C=N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-588 cm⁻¹ (C-Br)</td>
</tr>
</tbody>
</table>

Table 2: Tested strains and cultivation conditions.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Strains</th>
<th>Cultivation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-Positive bacteria</td>
<td><em>Staphylococcus aureus</em> (RCMB 000106)</td>
<td>Nutrient agar, 37ºc, 24-48h</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus subtilis</em> (RCMB 00107)</td>
<td></td>
</tr>
<tr>
<td>Gram-Negative bacteria</td>
<td><em>Pseudomonas aeruginosa</em> (RCMB 00102)</td>
<td>Nutrient agar, 37ºc, 24-48h</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> (RCMB 00103)</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Aspergillus fumigatus</em> (RCMB 002003)</td>
<td>Malt extract agar, 28 ºc, 48-72h</td>
</tr>
<tr>
<td></td>
<td><em>Geotrichum candidum</em> (RCMB 052006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Candida albicans</em> (RCMB 005002)</td>
<td></td>
</tr>
</tbody>
</table>

The used reference drugs in this study were Penicillin G and Streptomycin (30 ug/ml) anti-bacterial drugs, in addition to Itraconazole and Clotrimazole (30 ug/ml) as anti-fungal drugs.

All the tested compounds were dissolved in dimethylsulfoxide (DMSO) with final concentration of 10 µg/ml of each tested derivative and were tested in triplicate then the average inhibition zone diameters (mm) were determined.

Thin section transmission electron microscope (TEM) (JEOL 1010, Jeol Korea Ltd., South Korea) was carried out by the laboratory of electron microscope at the Regional Center for Mycology and Biotechnology, Al-Azher University, Cairo, Egypt.

**SYBR green-based real time-Polymerase Chain Reaction (rt-PCR):**

DNA of treated and untreated micro-organisms was extracted from bacterial and fungal colonies by using MagNA Pure Compact (Roche diagnostic, Germany) according to the method of Randegger and Hačhler (2001) and Espy et al., (2006).

The extracted DNA was used as a template for the SYBR green-based rt-PCR. The dsDNA-specific dye SYBR Green I has been used to analyze the melting curves of PCR products, which are characterized by a rapid loss of fluorescence as the temperature is raised through the samples’ melting temperature (Tm) (Ririe et al., 1997). PCR was performed for amplification of 16S rRNA gene in *Staphylococcus aureus* and large subunit rRNA gene in both *Geotrecum candidum* and *Candida albicans* using LightCycler Carousel-Based System (Roche diagnostic, Germany). Primers used are illustrated in table (3) (Haynes et al., 1995, Hilali et al., 1997).

Amplification was performed according to the following protocol: denaturation at 95 ºc for 60 sec for bacteria and 120 sec for fungi, amplification by performing 45 cycles each cycle consisted of three segments: denaturation at 95 ºc for 5 sec, annealing at 57ºc for 10 sec. and extension at 72 ºc for 15 sec. and after completion, the melting curve was recorded by cooling to 40 ºc at 20 ºc/sec, holding at 40 ºc for 30 sec, and then heating slowly at 0.2 ºc/sec until 85 ºc.
Table 3: Primer sequence and genes to be amplified.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Primer sequence</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. Aureus</td>
<td>F 5’ CAG CAG CGG CGG TAA TAC 3’</td>
<td>16S rRNA</td>
</tr>
<tr>
<td></td>
<td>R 5’ CCG TCA ATT CCT TGT AGT TT 3’</td>
<td></td>
</tr>
<tr>
<td>Geo. candidum</td>
<td>F 5’ GCA TAT CAA TAA GCG GAG GAA AAG 3’</td>
<td>large subunit RNA</td>
</tr>
<tr>
<td></td>
<td>R 5’ GGG CCG TGT TTC AAG ACG 3’</td>
<td></td>
</tr>
<tr>
<td>Candid. albicans</td>
<td>F 5’ TGT GAG CGG GAG GAT AAT GG 3’</td>
<td>large subunit RNA</td>
</tr>
<tr>
<td></td>
<td>R 5’ GGT CCG TGT TTG AAG ACG 3’</td>
<td></td>
</tr>
</tbody>
</table>

Molecular Docking:

All the molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE, 10.2008) software which provided by chemical computing group, Canada. Docking on the active site of gyrase was performed for synthesized compounds (Ia, Ib, II, III and IV). All the minimizations were performed with MOE until a root mean standard deviation (RMSD) gradient of 0.05 kcal mol$^{-1}$Å$^{-1}$ with MMFF94X force field and the partial charges were automatically calculated. The X-ray crystallographic structure of gyrase with its ligand (PDB ID: 3U2D) was obtained from the protein data bank. The enzyme was prepared for docking studies where: (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active site search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. (iv) The obtained model was then used in predicting the ligand enzyme interaction at the active site.

Results:

Antimicrobial Activity:

Table (4) illustrate that all compounds had no effect on *Pseudomonas aeruginosa*. Compounds II, III and IV were more potent against bacteria than the starting compound (Ia) since they produced inhibition zones larger than those produced by Ia. From MIC results, compound II was the most active since it affected tested bacteria with the minimum concentration (19µg/ml against G+ve and 156 µg/ml against G-ve strains). Data revealed that substituted quinazolinones were more potent against G+ve than G-ve. In addition, the tested quinazolinone derivatives were less potent than reference drugs.

With regard to fungal strains, compound III had no effects on all the tested strains, while compound II was more potent than compounds Ia and IV. The anti-fungal activities of the tested derivatives were close to those of clotrimazole.

Table 4: Zone of inhibition (mm) and minimum inhibitory concentration (MIC) values (µg/ml) of the tested derivatives with regard to reference drugs.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Gram +ve</th>
<th>Gram –ve</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aur. mm/MIC</td>
<td>B. sub. mm/MIC</td>
<td>P. aer. mm/MIC</td>
</tr>
<tr>
<td>Ia</td>
<td>13/78</td>
<td>14.2/78</td>
<td>NA</td>
</tr>
<tr>
<td>II</td>
<td>24.4/19</td>
<td>25.4/19</td>
<td>NA</td>
</tr>
<tr>
<td>III</td>
<td>20.4/39</td>
<td>21.8/78</td>
<td>NA</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>29.5/-</td>
<td>32.6/-</td>
<td>28.3/-</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>25/-</td>
<td>29/-</td>
<td>24/-</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

- NA: No Activity

TEM micrograph of *Staphylococcus aureus* (figure 1) showed that the bacterial cell was affected by all the tested compounds (II, III and IV) as compared to control. The most obvious difference was the rupture of the cell wall and shrinkage of the cytoplasm. In regard to *Bacillus subtilis* (figure 2), the bacterial cell was affected by all the tested compounds (II, III and IV) as compared to control. The most obvious differences in TEM micrograph (b) and (d) were the rupture of the cell wall and shrinkage of the cytoplasm, while in (c) deformation of the cell was observed and cytoplasm becomes denser.
Fig. 1: TEM micrograph of *Staphylococcus aureus* cells either untreated (a) or treated with compound II (b), treated with compound IV (c) or treated with compound III (d).

*Aspergillus fumagatus* mycelia cell wall treated with compound II was thicker than untreated cells. The cytoplasmic materials had been shrunk and the cell membrane was away from the cell wall leaving a large space in between (figure 3). In figure (4) derivative II also produced dramatic cellular changes in the mycelia of *Geotrecum candidum* where the cells of the treated fungus revealed deformed mitochondria as compared to untreated. In addition cytoplasm becomes thicker with internal lyses of its components.

Fig. 2: TEM micrograph of *Bacillus subtilis* cells either untreated (a), treated with compound II (b), treated with compound IV (c) or treated with compound III (d).

Fig. 3: TEM micrograph of mycelia (LS) of *Aspergillus fumagatus* either untreated (a) or treated with compound II (b).
Fig. 4: TEM micrograph of mycelia (LS) of *Geotrichum candidum* either untreated (a) or treated with compound II.

Figure (5) revealed that compound II produced many changes in the microscopically feature of *Candida albicans* cells. Treated cells appeared with irregular cell shape and asymmetric cell wall compared to control cells. The cytoplasm had been shrunk leaving a large distance from the cell wall as compared to untreated cells. In addition internal lyses appeared along the cell and the cytoplasmic granules became smaller than untreated cells.

**SYBR green-based rt-PCR:**

Figure (6) illustrated that derivative IV had no effect on *Staphylococcus aureus* 16S r-RNA gene, since its Tm was the same as untreated bacteria (78.84°C). Derivatives II and III were effective on the bacterial gene leading to change the Tm of the DNA to 76.69°C. Compound II revealed peaks at 69.87°C and 76°C or large subunit r-RNA genes of *Geotricum candidum* and *Candida albicans*, respectively which were lower than Tm of untreated DNA.

**Fig. 6:** Melting temperatures of the amplified genes in non treated and treated microorganisms.
**Molecular Docking Study:**

The protein data bank file (PDB ID: 3U2D) was selected for testing the molecular docking on the active site of gyrase enzyme. The file contains gyrase enzyme co-crystallized with a pyrolamide ligand. Docking protocol was verified by redocking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S) = −24.0919 kcal/mol and root mean standard deviation (RMSD) = 0.4236 (table 5, Fig. 7).

**Table 5:** Binding scores and amino acid interactions of the docked compounds on the active site of gyrase.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>S(kcal/mol)</th>
<th>Amino acid interactions</th>
<th>Interacting moieties</th>
<th>H-bond length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand*</td>
<td>−24.0919</td>
<td>Asp 81</td>
<td>NH pyrrole</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asp 81</td>
<td>C=O</td>
<td>2.72 (through a water molecule)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gly 85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thr 137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>−9.1588</td>
<td>Arg 144</td>
<td>C=O</td>
<td>2.51, 2.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg 144</td>
<td>C=O</td>
<td>2.96 (through a water molecule)</td>
</tr>
<tr>
<td>Ib</td>
<td>−4.0970</td>
<td>No interactions</td>
<td>No interactions</td>
<td>No interactions</td>
</tr>
<tr>
<td>II</td>
<td>−12.4481</td>
<td>Arg 144</td>
<td>C≡N</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg 144</td>
<td>C≡N</td>
<td>3.43 (through a water molecule)</td>
</tr>
</tbody>
</table>

* RMSD = 0.4236

The pyrolamide ligand interacts with the active site of gyrase by four interactions: Asp 81 with a hydrogen bond of 1.76 Å by NH of the pyrrol moiety, Asp 81, Gly 85 and Thr 137 through a water molecule with a hydrogen bond of 2.72 Å between the C=O of the ligand and the water molecule.

![Co-crystallized of the inhibitor on the active site of gyrase](image)

**Fig. 7:** Co-crystallized of the inhibitor on the active site of gyrase

Derivatives III and IV did not fit to the active site of gyrase, while compound Ib occupied the active site but with no interactions (Fig. 8). Energy scores (S) and amino acid interactions for compounds Ia, Ib and II were listed in Table (5). Compound Ia revealed energy score (S) = −9.1588 kcal/mol and interacted with Arg 144 with two hydrogen bonds of 2.51 and 2.75 Å through C=O of the pyrimidine ring and through a water molecule with a hydrogen bond of 2.96 Å also through its C=O (Fig. 9).
Fig. 8: Docking of compound Ia into the active site of gyrase.

Fig. 9: Docking of compound Ib into the active site of gyrase.

Compound II showed the best energy score \( S = -12.4481 \text{ kcal/mol} \) and interacted with Arg 144 with a hydrogen bond of 2.70 Å and through a water molecule with a hydrogen bond of 3.43 Å through its C≡N of the pyrimidine ring (Fig. 10 & 11).
Fig. 10: Docking of compound II into the active site of gyrase.

Fig. 11: 3D of compound II on the active site of gyrase.

**Discussion:**

The present study revealed that the synthesized quinazolinone derivatives (II, III and IV) were potent against Gram-positive bacteria since their inhibition zones were close to those produced by the reference drug streptomycin. The ranking of the three derivatives according to their activity was II, IV and III.

These results agree with those of Gupta *et al.*, (2008) who reported that quinazolinone derivatives exhibited interesting high activity against *Staphylococcus aureus* and *Bacillus subtilis*. Patel *et al.*, (2010) confirmed this antibacterial activity of quinazolinones containing pyrazoline moiety.

The studied three derivatives were unsubstituted at position 4 as their parent compound (I) and consequently possess free carbonyl oxygen. The structure activity relationship (SAR) of compound II demonstrates that substitution of hydrogen at position 3 of pyrimidine ring with ethyl acetate revealed great potency. So, the anti-bacterial activity of II may be due to the prevention of the keto-enol tautomerism unlike compounds III and IV. In addition, presence of active methylene at position 2, which considered as an electron releasing group, may also increase the activity of the derivative through the cyano moiety. Activity of derivative IV may be related to the presence of an amide group at position 2 of pyrimidine ring which possess chemical activity lower than cyan moiety. The activity of all the tested derivatives may also be due to the presence of another fused benzene ring since most effective quinazoline derivatives were reported to be those carrying an unsubstituted benzene ring or one substituted with small substituents while having pyrimidine ring substituted with larger substituents, such as phenyl moiety (Jantová *et al.*, 2004) as in derivative III.

This antibacterial activity of the tested derivatives against Gram +ve bacteria may be related to their ability to affect the permeability of the bacterial cell wall through interacting with the peptidoglycan layer and/or
teichoic acid since these two strains as all other Gram +ve bacteria possess a thick peptidoglycan layer formed from linear chains of amino sugars (N-acetyl glucosamine and N-acetyl muramic acid) (Ryan et al., 2010). These interactions produced a flux of protons which induces changes in cell wall and cell membrane and ultimately, cell death. This explanation was confirmed by the present results of TEM micrographs which showed rupture of the cell wall and cell membrane of the tested Gram-positive bacteria.

With regard to the effect of the tested derivatives on rRNA gene, compounds II and III affected Staphylococcus aureus 16S rRNA gene. The two tested derivatives decreased the DNA melting temperature (Tm) of treated bacteria (76.69°C) than that recorded by untreated control (78.84°C). On the other hand, compound IV showed no effect on the studied gene since its Tm was similar to that of untreated bacterial cell.

El-Azab et al. (2010) experiments illustrated that the N-aminoalkyl (anilino)-6,7-dimethoxyquinazoline nucleus is an efficient pharmacophore to trigger binding to DNA, via an intercalative binding process. The agreement between the three derivatives in the inhibition of bacterial growth could be correlated to a similar inhibitory mechanism related to the common structural feature in the three derivatives (the quinazolinone fragment) while the variation in the effect on DNA is probably caused by the differences in the substitution pattern in the three compounds at positions 2 and 3 of pyrimidine ring.

In addition, this decrease in melting temperature, in case of derivatives II and III, may indicate that there is disturbance either in nucleotide sequence or length.

The effect of studied derivatives on Gram –ve bacteria showed weak inhibitory activity against E. coli. Although these compounds affected the bacterial growth, their MICs were very high which indicate poor activity. In addition, zones of inhibition produced by these compounds were very small comparing to those produced by reference drugs.

The present results agree with those reported by other researchers (Kluytmans et al., 1997, Singhal et al., 2011) but disagree with results reported by Chandrika et al., (2009) who stated that their quinazolinone derivatives were potent against Gram-negative bacteria.

Although compound IV produced the largest zone of inhibition (11.2mm), its MIC was very high (625ug/ml), consequently, compound II (its zone of inhibition was 10.8mm) considered the most potent derivative since its MIC (156 ug/ml) was lowered than that of compound IV.

Gram-negative cell wall contains an outer membrane composed of phospholipids and lipopolysaccharides. These constituents increase the negative charge of the cell membrane and helps stabilize the overall membrane structure, which is of crucial importance to Gram-negative bacteria, whose death results if it is mutated or removed (Wang and Quinn, 2010). According to the chemical structure of studied derivatives, compound II considered more negatively charged than the rest derivatives due to the polarity on the cyano group that can disturb the electron distribution on the cell membrane.

Although peptidoglycan layer is thinner in Gram-negative than Gram-positive bacteria but the effects of the tested compounds were not as potent as in Gram-positive. This result confirms the suggestion that the main cause of the anti-bacterial effect of quinazolinone derivatives was due to their interaction with the teichoic acids (not found in Gram-negative bacterial cell wall), in addition to their effects on the peptidoglycan layer.

The present study revealed that compound II was the most potent quinazolinone derivative against fungal strains. This derivative possessed the lowest MIC and the largest zone of inhibition near to those produced by reference drugs. In regard to compound IV, it was moderately effective, while compound III had no anti-fungal effect. These results agree with results reported by many others (Aly 2007, Deshmukh et al., 2010, Boyapati et al., 2010, Patel et al., 2010).

The activity of compound II against fungi may be due to the presence of the highly negatively charged region of –CH2CN at position 2 beside carbonyl oxygen at position 4 in pyrimidine ring. The -CH2CN moiety can attack the chemical components of the cell wall by either direct interaction or by disturbing the electron balance of the cell wall leading to deformation of cell wall symmetry. This penetration exposed the cytoplasm to the exogenous media leading to its destruction.

This assumption confirmed by the TEM micrographs which revealed either increasing in the cell wall thickness in both Aspergillus fumigatus and Geotrichum candidum or rupture and irregularity in the cell wall of Candida albicans.

In addition, the melting curve analysis revealed that compound II affected gene codes for large subunit rRNA in both Geotrichum candidum and Candida albicans, since it decreased the Tm of DNA of treated fungi as compared to the untreated ones. This may be due to firstly, disturbance in the hydrogen bonds between the different base pairs as a result of the carbonyl oxygen and cyano group of compound II. Secondary, compound II replaced one or more of DNA nitrogenous bases, consequently disturbed the nucleotide sequence. This assumption confirmed by the recorded melting temperature of the treated gene which was lowered than untreated DNA. This reduction in melting temperature may be related to reduction in GC/AT ratio.

However compound IV does not resemble compound II chemically, and the amide group is not as stronger as cyano group but it can react as cyano moiety in attacking the components of the cell wall and DNA. This is
confirmed by the lowest zone of inhibition produced by this compound and higher MIC, compared to compound II.

Schnell et al., (2004) and Boyapati et al., (2010) reported that the effect of quinazolinone nucleus on bacterial cell may be due to either inhibition of DNA gyrase or inhibition of dihydrofolate reductase which are important for cell proliferation and cell growth. Docking studies were performed in this work to identify the nature and amount of interactions of the synthesized quinazoline derivatives with DNA-gyrase enzyme. For compound Ia, the keto-enol tautomerism enables oxygen in position 4 with its negative charge to attack the positively charged argenine residue and a molecule of water. Althuough this tautomeric effect was blocked in compound II, but the nitrogen of cyanide moiety in presence of the active methylene, which is electron releasing group, possessed high electron density which enabled it to attack both positively charged argenine residue and one molecule of water. The lower interaction energy observed for Ia rationalizes the insufficient binding into the gyrase active site than that of the compound II confirming the potent activity of compound II.

Conclusion:
Quinazolinone derivatives possess anti-bacterial activities, especially against the Gram positive strains, and anti-fungal strains through their interaction with the cell wall and DNA structures. In spite the tested compounds contain carbonyl oxygen attached directly to the pyrimidine ring, the type and position of chemical substituent confers the antimicrobial activities. This study is considered a spot of light of how quinazolinone derivatives act as antimicrobial agents.

REFERENCES


