Synthesis, Anti-microbial and Molecular Docking Studies of Quinazolin-4(3H)-one Derivatives

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Abstract: In this work, synthesis, antimicrobial activities and molecular docking studies of some new series of substituted quinazolinone 2a–h and 3a–d were described. Starting form 2-aminobenzamide derivatives 1, a new series of quinazolinone derivatives has been synthesized, in high yields, assisted by microwave and classical methods. Some of these substituted quinazolinones were tested for their antimicrobial activity against Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus, and Bacillus subtilis), and anti-fungal activity against (Aspergillus fumigatus, Saccharomyces cerevisiae, and Candida albicans) using agar well diffusion method. Among the prepared products, 3-benzyl-2-(4-chlorophenyl)quinazolin-4(3H)-one (3a) was found to exhibits the most potent in vitro anti-microbial activity with MICs of 25.6 ± 0.5, 24.3 ± 0.4, 30.1 ± 0.6, and 25.1 ± 0.5 µg/mL against Staphylococcus aureus,
**Bacillus subtilis**, *Pseudomonas aeruginosa* and *Esherichia coli*, respectively. Compound 3a was found to exhibit the most potent *in vitro* anti-fungal activity with MICs of 18.3 ± 0.6, 23.1 ± 0.4, and 26.1 ± 0.5 µg/mL against *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Candida albicans*, respectively.

**Keywords:** quinazolinone; antimicrobial agents; streptomycin; clotrimazole; molecular docking

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### 1. Introduction

In the last decade, quinazolines have been extensively studied in medicinal chemistry. The quinazolinone scaffold is considered to be a motif structure. Quinazolinone ring system is found in a variety of bioactive natural as well as synthetic products. Many natural products contain quinazolinone core structures for example asperlicin C, sclerotigenin, circumdatin F, benomalvin A, and many others have been documented as biologically important molecules [1–3]. Some synthetic quinazolinones, such as ispinesib, raltitrexed, halofuginone, tempostatin, etc. have been in the market or are currently in clinical trials for various cancer treatments. Quinazolines have exhibited therapeutic activities including antibacterial [4,5], antiviral [6], antifungal [7,8], antimalarial [9], antihypertensive [10], anticancer [11–13], diuretic [14,15], inhibition of derived growth factor receptor phosphorylation [16], antagonism of ghrelin receptor [17], anticonvulsant [18], COX-2 inhibitory activities [19,20] analgesic and anti-inflammatory.

Particularly, enzyme Sortase A involves in the pathogenesis of variety of bacterial infections, including respiratory tract, bloodstream, skin and tissue infection which serve to anchor some proteins responsible for virulence mainly by Gram +ve bacteria. Sortase A has been attracted great interest as potential drug targets since decades [21]. The inhibition of *Sortase A* activity results in the separation of *S. aureus* from the host cells and ultimately alleviation of the infection. We used these newly synthesized active inhibitors to explore the binding cavity of *Staphylococcus aureus* Sortase A using GOLD docking program [21].

Recently, Mabkhot and co-workers have been involved in a research program aimed to development of new synthetic strategies for novel bioactive molecules and evaluation of their biological activity [22–28].

In this paper, we synthesized some new substituted quinazolinones as potential anti-microbial agents starting form 2-aminobenzamide derivatives. The validity of this hypothesis was confirmed through preliminary *in vitro* anti-bacterial and anti-fungal screening of the desired molecules and their molecular docking studies.
2. Results and Discussion

2.1. Chemistry

Synthesis of the desired compounds 2a–h was achieved by allowing 2-aminobenzamide derivatives 1a–h [29,30] to undergo ring closure either with triethyl orthoformate under classical reflux condition as shown in Scheme 1. Alternatively, 2a–f, h can be obtained by microwave mediated methodology. It is proposed that the product 2a–h was formed via initial nucleophilic addition of amide group into electrophilic carbon followed ring closure and elimination of three molecules of EtOH to give the desired products 2a–h. Assignment of structures of 2a–h is based on elemental analysis and spectral data. Their IR spectra showed the disappearance of the characteristic absorption bands of NH & NH$_2$ groups. Their $^1$H-NMR spectra showed in each case a characteristic singlet assigned to proton of N=CH. Their MS spectra are matched with the designed structures.

Scheme 1. Synthesis of quinazolin-4(3H)-one derivatives 2a–h from 2-aminobenzamide derivatives 1a–h.

Next, compounds 3a–d were synthesized by reaction of 2-aminobenzamide derivatives 1a–d with p-chlorobenzaldehyde, in DMF under reflux for 12 h, as shown in Scheme 2. On the other hand, microwave mediated methodology gave the same 3a,b. It is assumed that the products 3a–d
were formed via initial nucleophilic addition of amide to carbonyl group followed ring closure and elimination of H₂O to give the desired products 3a–d. Elucidations of the chemical structures of 3a–d are inferred from their spectroscopic and analytical data. Their IR spectra showed the disappearance of the characteristic absorption bands of NH & NH₂ groups. The NMR and MS spectra are matched with the designed structures.

Scheme 2. Synthesis of quinazolin-4(3H)-one derivatives 3a–d from 2-aminobenzamide derivatives 1a–d.

2.2. Antimicrobial Evaluation

Antimicrobial activity of the synthesized compounds has been screened against micro-organisms representing Gram-(+ve) bacteria (Staphylococcus aureus and Bacillus subtilis), Gram-(−ve) bacteria (Escherichia coli and Pseudomonas aeruginosa), and fungi (Aspergillus fumigatus, Saccharomyces cerevisiae and Candida albicans), using the bioassay technique of antibiotics as antibacterial and
antifungal standard drug specified in the US Pharmacopeia at 25 µg/mL. Among the screened compounds, only compound 3a showed a potent inhibitory effect against Gram-(+ve) bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram-(−ve) bacteria (Escherichia coli and Pseudomonas aeruginosa). Compound 3a found to be as similar potency as the standard drug (streptomycin). Nevertheless, 3a showed the strong inhibitory effect against fungi (Saccharomyces Cerevisiae, Aspergillus fumigatus and Candida albicans). The results indicate that compounds 3a has excellent biological activity and can be subjected for further evaluation for example enzyme inhibition. Clotrimazole and Streptomycin were used as standards [31]. The results obtained are listed in Table 1.

Table 1. Antimicrobial evaluation of the synthesized molecules.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Gram-Postive Bacteria</th>
<th>Gram-Negative Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>7.8 ± 0.4</td>
<td>9.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>12.3 ± 0.3</td>
<td>13.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>9.8 ± 0.3</td>
<td>12.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>2g</td>
<td>10.3 ± 0.2</td>
<td>11.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td>9.1 ± 0.4</td>
<td>9.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>25.6 ± 0.5</td>
<td>24.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>10.4 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>clotrimazole</td>
<td>25.6 ± 0.5</td>
<td>24.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>streptomycin</td>
<td>25.6 ± 0.5</td>
<td>24.3 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

2.3. Molecular Docking Studies

The antimicrobial potency of all the newly synthesized compounds were subjected for further docking studies to explore the binding pattern against methicillin resistant Staphylococcus aureus (MRSA) [21]. PDB (ID 1T2W) [32] with 1.80 Å resolution was retrieved from Brookhaven Protein Data Bank. From our previous studies residues including Ala92, Ala104, Glu105, Ala118, Pro163, Leu169, Gln172, Thr180, Ile182, Trp194 and Ile199 were found to be in the active site of the receptor, responsible for hydrophobic interactions. While Arg197 was the hotspot residue showing significant hydrogen bond within the binding pocket [21]. Conformational search of ligands were investigated via Gold docking program with extensive genetic algorithm. In this study, ten conformers were generated for each ligand using default parameters. Docking of all the newly synthesized inhibitors showed hydrogen bond interactions with Arg197 mentioned in Figure 1, supported our by previous findings [21]. The most active newly synthesized compound as an antimicrobial agent 3a showed potent inhibitory activity; involve in hydrogen bonding interaction with oxygen of quinazoline moiety at 2.13 Å with Arg197. While the hydroxyl group of Ser116 showed strong hydrogen bond interaction with Ser116 at a bond distance of 2.29 Å and chloro moiety of benzene ring attached to quinazoline showed hydrogen bond with Ala92 with a distance of 2.64 Å. Hydrophobic interaction also observed between Trp194 and first benzene ring of the quinazoline moiety (Figure 2).
Figure 1. Docked ligand at the receptor binding site. This picture represent 2D-interactions for the newly synthesized inhibitors within the binding pocket of target receptor using Poseview.

Figure 2. Molecular Docking Interaction diagram for the most potent compound 3a. Panel (a) is a two-dimensional representation of the docked pose by Poseview. Panel (b) representing three-dimensional view by MOE.
3. Experimental Section

General: The \(^1\)H-NMR and \(^\text{13}\)C-NMR spectra were run in DMSO-\(d_6\) and recorded on a Varian Mercury or Jeol-400 NMR spectrometer. Coupling constants \(J\) are given in Hz and chemical shifts (\(\delta\)) are referred in ppm and related to that of the solvent. Abbreviations for multiplicity are as follows: m (multiplet), q (quadruplet), t (triplet), d (doublet) and s (singlet). IR spectra were recorded on a Perkin Elmer FT 1000 spectrophotometer using KBr pellets. Mass spectroscopy was measured on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Melting points were measured on a Gallenkamp melting point apparatus in open glass capillaries and are uncorrected. Elemental analysis was carried out on an Elementar Vario EL analyzer.

3.1. General Procedure for the Preparation of Quinazolin-4(3H)-one Derivatives 2a–h

**Method A (2a–h):** To a mixture of 2-aminobenzamide derivatives 1a–h (2 mmol) was added triethyl orthoformate (2–3 mL) and the reaction mixture was heated under reflux for 12 h. The reaction was monitored by TLC (EtOH: CHCl\(_3\)), then left to cool to RT. The precipitated solid was filtered off, washed with diethyl ether and recrystallized from the proper solvent to afford the corresponding product 2a–h.

**Method B (2a–f, h):** A mixture of 2-aminobenzamide derivatives 1a–g, h (2 mmol) and triethyl orthoformate (5–10 mmol) in the presence of view drops of DMF, was exposed to microwave irradiation (140–420 w) for 4–10 min. The reaction mixture was left to cool to RT and methanol was added. The formed solid product was filtered off, washed with diethyl ether and recrystallized from the appropriate solvent to afford the corresponding product 2a–g, h.
3-Benzylquinazolin-4(3H)-one (2a). This compound was prepared from 2-amino-N-benzylbenzamide 1a according to method A or method B (4 min, 280 W), and recrystallized from ethanol afford 2a as shining white needles; yield (82a, 65b%); m.p. 116 °C; IR νmax (KBr) 1630.75, 1578.88 cm⁻¹; 1H-NMR (400 MHz, DMSO-d₆) (ppm): δ 8.58 (1H, s), 8.16 (1H, d, J = 8.0 Hz), 7.84 (1H, t, J = 8.0 Hz), 7.70 (1H, d, J = 8.0 Hz), 7.55 (1H, t, J = 8.0 Hz), 7.28–7.38 (5H, m), 5.20 (2H, s, CH₂); 13C-NMR: δ 50.0, 122.20, 126.68, 127.77, 127.83, 128.21(2C), 128.24, 129.21(2C), 135.00, 137.42, 148.48, 148.58, 160.7; MS m/z (%): 236[M⁺](C₁₅H₁₂N₂O); Anal. For C₁₅H₁₂N₂O (236.27) calcd; C, 76.25; H, 5.12; N, 11.86; Found: C, 76.24; H, 5.12; N, 11.86.

3-(3,4-Dimethoxyphenyl)quinazolin-4(3H)-one (2b). Prepared from 2-amino-N-(3,4-dimethoxyphenyl)benzamide (1b) according to method A or method B (6 min, 280 W), and recrystallized from ethanol to afford 2b, dark powder; yield (99a, 70b%); m.p. 140 °C; IR νmax (KBr) 1669.14, 1595.03 cm⁻¹; 1H-NMR (400 MHz, DMSO-d₆) (ppm): δ 8.31(1H, s), 8.20 (1H, d, J = 8.0 Hz), 7.88 (1H, t, J = 8.0 Hz), 7.74 (1H, d, J = 8.0 Hz), 7.60 (1H, t, J = 8.0 Hz), 7.18 (1H, s), 7.1 (1H, d, J = 8.0 Hz), 7.06 (1H, d, J = 8.0 Hz), 3.77, 3.83 (each 3H, s, 2 (OCH₃)); 13C-NMR: δ 25.3, 34.9, 55.9, 56.0, 112.37, 113.07, 120.93, 121.09 (2C), 121.65, 123.00, 128.47, 132.24, 132.32, 139.36, 147.78, 149.10, 168,7; MS m/z (%): 282[M⁺](C₁₆H₁₄N₂O₃) (26.56), 222 (69.23), 193 (51.56), 193 (50.77), 43 (80.00), 55 (100); Anal. for C₁₆H₁₄N₂O₃ (282.29) calcd; C, 68.07; H, 5.00; N, 9.92; Found: C, 68.10; H, 5.03; N, 9.91.

3-(3,4-Dimethoxyphenethyl)quinazolin-4(3H)-one (2c). Prepared from 2-amino-N-(3,4-dimethoxyphenethyl)benzamide 1c according to method A, as beige powder; yield (95a%); m.p. 140 °C; IR νmax (KBr) 1681.65, 1642.74 cm⁻¹; 1H-NMR (400 MHz, DMSO-d₆) (ppm): δ 8.76 (1H, s), 8.36 (1H, d, J = 7.8 Hz), 7.65 (1H, d, J = 7.8 Hz), 7.46 (1H, t, J = 7.8 Hz), 3.69 (2H, s, CH₂); 13C-NMR: δ 25.3, 34.9, 55.9, 56.0, 112.37, 113.07, 120.93, 121.09 (2C), 121.65, 123.00, 128.47, 132.24, 132.32, 139.36, 147.78, 149.10, 168,7; MS m/z (%): 310[M⁺](C₁₈H₁₈N₂O₃) (15.62), 293 (100), 275 (35.94), 28 (21.87), 44 (20.31), 69 (18.75); Anal. for C₁₈H₁₈N₂O₃ (310.35) calcd; C, 69.66; H, 5.85; N, 9.03; Found: C, 69.64; H, 5.83; N, 9.05.

2-(4-(4-Oxoquinazolin-3(4H)-yl)phenyl)acetic Acid (2d). Compound 2d was prepared from 2-(4-(2-aminobenzamido)phenyl)acetic acid 1d according to method A or method B (5 min, 280 W), and recrystallized from ethanol afford 2d as, brownish powder; yield (85a, 70b%); m.p. 267 °C; IR νmax (KBr) 3678.56, 3449.51, 1687.32, 1611.24, 1564.26 cm⁻¹; 1H-NMR (400 MHz, DMSO-d₆) (ppm): δ 8.35 (1H, s), 8.21 (1H, d, J = 7.7 Hz), 7.89 (1H, t, J = 7.7 Hz), 7.75 (1H, d, J = 7.7 Hz), 7.60 (1H, t, J = 7.7 Hz), 7.49 (2H, d, J = 8.0 Hz), 7.45 (2H, d, J = 8.0 Hz), 3.69 (2H, s, CH₂); 13C-NMR: δ 25.3, 122.46, 127.01, 127.79 (2C), 127.89, 127.98, 130.81(2C), 135.23, 136.35, 136.63, 147.78, 148.29, 160.6, 173.1; MS m/z (%): 280[M⁺](C₁₆H₁₂N₂O₃) (100), 250 (19.05), 235 (92.55), 207 (15.24),129 (32.38), 107 (56.19); Anal. for C₁₆H₁₂N₂O₃ (280.28) calcd; C, 68.56; H, 4.32; N, 9.99; Found: C, 68.55; H, 4.31; N, 10.01.

3-(4-Methoxyphenyl)quinazolin-4(3H)-one (2e). Compound 2e was prepared from 2-amino-N-(4-methoxyphenyl)benzamide 1e according to method A or method B (4 min,420 W), and recrystallized from ethanol afford 2e as shining white needles; yield (99a, 95b%); m.p. 195 °C; IR νmax (KBr)
3-(4-Chlorobenzyl)quinazolin-4(3H)-one (2f). Compound 2f was prepared from 2-amino-N-(4-chlorobenzyl)benzamide 1f according to method A or method B (2 min, 280 W) and recrystallized from methanol afford 2f as white powder; yield (88\(^a\), 62\(^b\))% m.p. 155 °C; IR \(\nu_{\text{max}}\) (KBr) 1602.24, 1531.10 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO-\(\text{d}_6\)) (ppm): \(\delta\) 8.60 (1H, s), 8.15 (1H, d, \(J = 8.0\) Hz), 7.83 (1H, t, \(J = 8.0\) Hz), 7.70 (1H, d, \(J = 8.0\) Hz), 7.31–7.41 (4H, m), 6.56 (1H, t, \(J = 8.0\) Hz), 5.19(2H, s, CH\(_2\)); \(^{13}\)C-NMR: \(\delta\) 49.9, 126.66, 127.82, 128.74, 150.10, 129.15 (2C), 129.57, 130.23 (2C), 132.40, 132.88, 135.07, 136.40, 148.5; MS \(m/z\) (%): 270 [M\(^+\)](C\(_{15}\)H\(_{11}\)N\(_2\)O\(_2\Cl)); Anal. for C\(_{15}\)H\(_{11}\)ClN\(_2\)O (270.71) calcd; C, 66.55; H, 4.10; Cl, 13.10; N, 10.35; Found: C, 66.54; H, 4.11; Cl, 13.09; N, 10.33.

3-(3,4,5-Trimethoxyphenyl)quinazolin-4(3H)-one (2g). Compound 2g was prepared from 2-amino-N-(3,4,5-trimethoxyphenyl)benzamide 1g according to method A or method B (4 min, 280 W), as brownish powder; yield (98\(^a\), 88\(^b\))% m.p. 115 °C; IR \(\nu_{\text{max}}\) (KBr) 1680.00, 1607.17 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO-\(\text{d}_6\)) (ppm): \(\delta\) 8.34 (1H, s), 8.21 (1H, d, \(J = 8.0\) Hz), 7.83 (1H, t, \(J = 8.0\) Hz), 7.70 (1H, d, \(J = 8.0\) Hz), 7.17 (1H, s), 6.92 (1H, s), 3.77,3.79 ( each 3H, s, 2(OCH\(_3\))), 3.76 (3H, s, OCH\(_3\)); \(^{13}\)C-NMR: \(\delta\) 56.3, 56.7, 106.11 (2C), 122.49, 126.97, 127.84, 150.10, 129.15 (2C), 129.57, 130.23 (2C), 132.40, 132.88, 135.07, 136.40, 148.5; MS \(m/z\) (%): 312 [M\(^+\)](C\(_{17}\)H\(_{16}\)N\(_2\)O\(_4\)) (6.25), 297 (31.25), 194 (100), 165 (25.00), 87 (17.18), 68 (23.44); Anal. for C\(_{17}\)H\(_{16}\)N\(_2\)O\(_4\) (312.32) calcd; C, 65.38; H, 5.16; N, 8.97; Found: C, 65.39; H, 5.15; N, 8.93.

8-Chloro-3-(4-methoxyphenyl)quinazolin-4(3H)-one (2h). 2h was prepared from 2-amino-3-chloro-N-(4-methoxyphenyl)benzamide 1h according to method A, or method B(4 min, 280 W) as shining beige powder; yield (97\(^a\), 60\(^b\))% m.p. 175 °C; IR \(\nu_{\text{max}}\) (KBr) 1631.68, 1610.34 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, DMSO-\(\text{d}_6\)) (ppm): \(\delta\) 8.43 (1H, s), 7.57–7.62 (3H, m), 7.43 (1H, d, \(J = 8.0\) Hz), 6.92 (2H, d, \(J = 8.8\) Hz), 6.66 (1H, t, \(J = 8.0\) Hz), 3.74 ( 3H, s, OCH\(_3\))); \(^{13}\)C-NMR: \(\delta\) 55.74, 114.26 (2C), 114.94, 115.90, 118.17, 119.48, 122.88 (2C), 128.21, 129.21, 132.42, 145.53, 156.21, 167.30; MS \(m/z\) (%): 28[M\(^+\)](C\(_{15}\)H\(_{11}\)N\(_2\)O\(_2\Cl)); 288 [M\(^+2\)] (C\(_{15}\)H\(_{11}\)N\(_2\)O\(_2\Cl)) (21.02), 187 (100), 244 (50.94), 43 (39.00), 189 (33.10); Anal. for C\(_{15}\)H\(_{11}\)ClN\(_2\)O\(_2\) (286.71) calcd; C, 62.84; H, 3.87; Cl, 12.37; N, 9.77; Found: C, 62.85; H, 3.87; Cl, 12.36; N, 9.78.

3.2. General Method for Preparation of Compounds Derivatives 3a–o

Method A (3a–d): To a solution of 2-aminobenzamide derivatives 1a–d (2 mmol) in DMF (2–3 mL), and \(p\)-chlorobenzaldehyde (2 mmol) was added. The reaction mixture was heated under reflux for 12 h. The reaction mixture was monitored by TLC (EtOH/CH\(_2\)Cl\(_2\)), then left to cool to RT, and poured...
into cold water (50 mL), the precipitated solid product was filtered off, and dried to afford the corresponding product 3a–d. **Method B (3a,b):** A mixture of 2-aminobenzamide derivatives 1a,b (2 mmol) and p-chlorobenzaldehyde (2 mmol) in the presence of view drops from DMF, was exposed to microwave irradiation (420–560 w) for 5–10 min. The reaction mixture was left to cool to RT. Water was added and the formed solid product was filtered off afforded the corresponding product 3a,b.

3-Benzyl-2-(4-chlorophenyl)quinazolin-4(3H)-one (3a). **3a** was prepared from 2-amino-N-benzylbenzamide 1a according to method A, or method B (5 min, 420 W) as white scales; yield (82a, 63b%); m.p. 101 °C; IR νmax (KBr) 1617.23, 1589.98 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d6) (ppm): δ 7.85 (1H, d, J = 8.0 Hz), 7.79 (2H, d, J = 8.8 Hz), 7.51–7.56 (3H, m), 7.25–7.36 (7H, m), 4.49 (2H, s, CH2); ¹³C-NMR: δ 40.3, 119.76, 126.81, 127.45 (2C), 128.02 (2C), 128.89 (2C), 129.52 (2C), 129.98, 131.14 (2C), 132.16, 134.84, 137.12, 139.61, 149.24, 161.74, 166.5; MS m/z (%): 346 [M+] (C21H15N2OCl) (100%), 347 [M++1] (23.00), 348[M++2] (33.61), 349 [M++3] (6.90%), 155 (60.55%), 91 (78.03%); Anal. for C21H15ClN2O (346.81) calcd: C, 72.73; H, 4.36; Cl, 10.22; N, 8.08; Found: C, 72.75; H, 4.36; Cl, 10.23; N, 8.10.

2-(4-Chlorophenyl)-3-(3,4-dimethoxyphenyl)quinazolin-4(3H)-one (3b). **3b** was prepared from 2-amino-N-(3,4-dimethoxyphenyl)benzamide 1b according to method A, or method B (5 min, 560 W) as shining pale green scales; yield (87a, 77b%); m.p. 147 °C; IR νmax (KBr) 1615.17, 1561.63 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d6) (ppm): δ 8.04(2H, d, J = 8.0 Hz), 7.92 (1H, d, J = 8.0 Hz), 7.60–7.65 (3H, m), 7.38–7.41 (3H, m), 7.33 (1H, d, J = 8.0 Hz), 6.91 (1H, t, J = 8.0 Hz), 3.70, 3.72 (each 3H, s, (2(OCH3)); ¹³C-NMR: δ 55.8, 56.3, 111.95, 112.61, 119.89, 126.99, 129.60 (2C), 129.77, 130.09, 131.22 (2C), 132.55, 132.89, 134.97, 137.42, 145.62, 149.11,149.20, 162.04, 164.6; MS m/z (%): 392 [M+] (C22H17N2O3Cl) (20.90%), 393 [M++1] (5.34), 391[M–H] (7.21), 219 (100), 91 (9.40), 64 (6.25); Anal. for C22H17ClN2O3 (392.83) calcd: C, 67.26; H, 4.36; Cl, 9.02; N, 7.13; Found: C, 67.13; H, 4.35; Cl, 9.08; N, 7.14.

2-(4-Chlorophenyl)-3-(3,4-dimethoxyphenethyl)quinazolin-4(3H)-one (3c). **Compound 3c** was prepared from 2-amino-N-(3,4-dimethoxyphenethyl)benzamide 1c according to method A, beige cubes; yield (78a%); m.p. 150 °C; IR νmax (KBr) 1618.50, 1614.63 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d6) (ppm): δ 7.65 (1H, d, J = 8.0 Hz), 7.34–7.44 (5H, m), 7.33 (1H, d, J = 8.0 Hz), 6.91 (1H, t, J = 8.0 Hz), 3.70, 3.72 (each 3H, s, (2OCH3)); ¹³C-NMR: δ 55.8, 56.3, 111.95, 112.61, 119.89, 126.99, 129.60 (2C), 129.77, 130.09, 131.22 (2C), 132.55, 132.89, 134.97, 137.42, 145.62, 149.11,149.20, 162.04, 164.6; MS m/z (%): 392 [M+] (C22H17N2O3Cl) (20.90%), 393 [M++1] (5.34), 391[M–H] (7.21), 219 (100), 91 (9.40), 64 (6.25); Anal. for C22H17ClN2O3 (392.83) calcd: C, 67.26; H, 4.36; Cl, 9.02; N, 7.13; Found: C, 67.13; H, 4.35; Cl, 9.08; N, 7.14.

2-(4-Chlorophenyl)-3-(3,4-dimethoxyphenethyl)quinazolin-4(3H)-one (3d). **Compound 3d** was prepared from 2-amino-N-(3,4-dimethoxyphenethyl)benzamide 1c according to method A, beige cubes; yield (78b%); m.p. 150 °C; IR νmax (KBr) 1618.50, 1614.63 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d6) (ppm): δ 7.65 (1H, d, J = 8.0 Hz), 7.34–7.44 (5H, m), 7.20 (1H, t, J = 8.0 Hz), 6.83 (1H, d, J = 8.4 Hz), 6.75 (1H, s), 6.68 (1H, t, J = 8.0 Hz), 6.63 (1H, d, J = 8.4 Hz), 3.70, 3072 (each 3H, s, 2(OCH3)), 2.96 (2H, t, J = 6.9 Hz, CH2), 2.65 (2H, t, J = 7.3 Hz, CH2); ¹³C-NMR: δ 20.0, 35.0, 47.5, 55.8, 112.39, 112.97 (2C), 114.82,115.43, 117.85, 121.03 (2C), 128.00, 128.73 (2C), 129.09 (2C), 131.91, 133.55, 133.86, 140.62, 146.73, 147.80, 149.10, 162.7; MS m/z (%): 168[M]+(C24H21N2O3Cl); Anal. for C24H21ClN2O3 (420.89) calcd: C, 68.49; H, 5.03; Cl, 8.42; N, 6.66; Found: C, 68.51; H, 5.02; Cl, 8.40; N, 6.68.

2-(4-(2-(4-Chlorophenyl)-4-oxoquinazolin-3(4H)-yl)phenyl)acetic Acid (3d). Compound 3d was prepared from 2-(4-(2-aminobenzamido)phenyl)acetic acid 1d according to method A, shining beige needles; yield (65b%); m.p. 213 °C; IR νmax (KBr) 3653.46, 3109.55, 1707.22, 1665.45, 1620.90 cm⁻¹; ¹H-NMR (400 MHz, DMSO-d6) (ppm): δ 12.32 (1H, s, OH), 8.00 (1H, d, J = 7.5 Hz), 7.71(1H, t,
J = 7.5 Hz), 7.60–7.66 (4H, m), 7.32 (2H, d, J = 7.3 Hz), 7.28 (1H, t, J = 7.5 Hz), 7.21 (2H, d, J = 7.3 Hz), 3.34 (2H, s, CH₂); 13C-NMR: δ 20.0, 115.43, 115.87, 120.06, 126.47(2C), 128.97(2C), 129.77, 130.00(2C), 131.17, 133.17(2C), 133.47, 134.43, 139.70, 140.33, 146.86, 162.70, 173.1, 173.4; MS m/z (%): 390[M+] (C₂₂H₁₅N₂O₃Cl) (3.12), 389 [M+ - C₂₂H₁₅ClN₂O₃ (390.82) calcd; C, 67.61; H, 3.87; Cl, 9.07; N, 7.17; Found: C, 67.60; H, 3.90; Cl, 9.10; N, 7.18.

3.3. Antimicrobial Evaluation [30]

3.3.1. Antifungal Activity

Samples of the synthesized molecules were subjected separately in vitro for their antifungal evaluation viz. Aspergillus fumigatus (RCMB 002003), Geotrichum candidum (RCMB 052006) Candida albicans (RCMB 005002) and Syncephalastrum racemosum (RCMB 005003). The culture of fungi was purified by single spore isolation technique. The antifungal activity was tested by agar well diffusion method according to the following procedure:

Sabourad dextrose agar plates: A homogeneous mixture of glucose-peptone-agar (40:10:15) was sterilized by autoclaving at 121 °C for 20 min. The sterilized solution (25 mL) was poured in each sterilized petri dish in a laminar flow hood and left for 20 min. to form the solidified sabourad dextrose agar plate. These plates were inverted and kept at 30 °C in an incubator to remove the moisture and to check for the contamination.

3.3.2. Antifungal Assay

Fungal strain was grown in 5 mL sabourad dextrose broth (glucose/peptone; 40:10) for 3–4 days to achieve 105 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the sabourad dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min. so that culture is properly adsorbed on the surface of sabourad dextrose agar plates. Small wells of size (4 mm × 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 μL of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds dissolved in DMSO were loaded as control. The plates were kept for incubation at 30 °C for 3–4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Clotrimazole and itraconazole were used as antifungal standard drugs.

3.3.3. Antibacterial Activity

Antibacterial evaluation was tested using agar well diffusion method. The activity of tested samples was tested against Staphylococcus aureus (RCMB 000106) and Bacillus subtilis (RCMB 000107), as Gram positive bacteria and Pseudomonas aeruginosa (RCMB 000102) and Escherichia coli (RCMB 000103), as gram negative bacteria. The solution of 5 mg/mL of each compound in DMSO was used for testing against bacteria. Centrifuged pellets of bacteria from 24 h old culture containing approximately 104–106 CFU (colony forming unit) per mL were spread on the surface of nutrient agar (ypetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1000 mL of distilled water, pH 7.0) and
was autoclaved under 121 °C for at least 20 min. Wells were created in medium with the help of sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 μL of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds were prepared in DMSO, and were loaded as control. The plates were kept for incubation at 37 °C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacteria. Streptomycin was used as an antibacterial standard drug.

3.3.4. Material and Methods

To investigate the binding pattern of the Quinazoline derivatives respect to their binding affinity, docking experiments were performed using genetic algorithm approach implemented in the GOLD [33] docking program for conformational search during docking process. PDB ID 1T2W retrieved from Protein Data Bank [34] and the co-crystallized ligand LPXTG peptide complex along with water molecules were omitted from the targeted protein. Default parameters were set to optimize the docking experiment. All the Ligands were sketched by ChemDraw Ultra and converted into 3D format using OpenEye Babel [35]. Hydrogen’s were added and MMFF94 partial charges were assigned to all ligands by MOE [36] prior to minimization. Furthermore, protonation states were corrected using OpenEye Filter [37] program before docking runs. MOE and Poseview [38] were used for the molecular interaction analysis.

4. Conclusions

We have successfully prepared quinazolin-4(3H)-one derivatives 2a–h and 3a–d starting from 2-aminobenzamide derivatives 1 with triethyl orthoformate and p-chlorobenzaldehyde, respectively, by one pot synthesis assisted by microwave or classical methods. The simple procedure, mild conditions, high yields and especially environmental friendliness make this protocol very attractive. Compound 3a showed strong inhibitory effect against Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus, and Bacillus subtilis), in addition to and anti-fungal activity against (Aspergillus fumigatus, Saccharomyces cerevisiae, and Candida albicans).

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Author Contributions

Yahia N. Mabkhot designed the study, carried out the synthesis. Assem Barakat wrote some research and audit. Munirah S. Al-Har and Fahad D. Aldawsari students did the experiments. Ali Aldalbahi edited the English language. Zaheer-Ul-Haq carried out molecular docking studies.
Conflicts of Interest

The authors declare no conflict of interest.

References and Notes


34. RCSDD PDB (Protein Data Bank) Homepage. Available online: http://www.rcsb.org/pdb (accessed on 1 January 2014).


37. OpenEye Scientific Software. *OE FILTER version 2.5*; OpenEye Scientific Software Inc.: Santa Fe, NM, USA.


*Sample Availability*: Samples of the compounds 2a–h and 3a–d are available from the authors.

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