Synthesis and Antibacterial Activity of 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazin-4-one and 3-amino-7-chloro-2-methyl quinazolin-4(3H)-one

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ABSTRACT
Quinazolines and quinazolinones are common structural motifs found in naturally occurring heterocycles. The current study is aimed at the synthesis and antibacterial evaluation of quinazolinone derivatives. The condensation of Methyl-2-amino-4-chlorobenzoate with acetic anhydride yielded the cyclic compound 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazin-4-one (1) which further produced 3-amino-2-methyl-7-chloro quinazolin-4(3H)-one (2) via the reaction with hydrazine hydrate. The structures of the synthesized compounds were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance (1H and 13C), Gas Chromatography-Mass Spectrometry and Elemental analysis. The synthesized compounds were screened for their antibacterial activity against various strains of bacteria: Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Serratia marcescens. Compounds 1 and 2 showed significant activity against Staphylococcus aureus and Serratia marcescens with MIC ranging from 6 – 12 mg/mL.

Keywords: 3-amino-7-chloro-2-methyl quinazolin-4(3H)-one, 7-chloro-2-methyl-4H-benzo [d] [1,3]-oxazin-4-one, Nucleophile, Quinazoline, Quinazolinone.

INTRODUCTION
Quinazoline is a bicyclic compound consisting of a pyrimidine system fused at 5, 6 with benzene ring having broad spectrum of medicinal values such as antibacterial,1, 2 anti-cancer,3 and anti-tubercular activities.4 Quinazolines and quinazolinones are common structural motifs found in naturally occurring heterocycles.5–7 Indeed, with particular reference to the pharmaceutical industry, heterocyclic motifs are especially prevalent with over 60% of the top retailing drugs containing at least one heterocyclic nucleus as part of the overall topography of the compound.8 The first quinazoline alkaloid to be isolated was vasicine (peganine 1) in 1888, produced by Indian medicinal tree Adhatoda vasica and later isolated from other species along with the quinazoline alkaloids, vasicinein 2 and deoxyvasicinine 3,9

In a quest to find additional quinazoline-based potential drugs, various substituted quinazolines have been synthesized which led to the synthesis of the derivative, 2-methaqualone. Methaqualone was synthesized for the first time in 1951 and it is the most well-known synthetic quinazoline drug famous for its sedative hypnotic effects.9 The structural diversity of quinazolines has been broadened with the discovery of asperlin along with asperlicins B, C, D and E.10, 11 The broad spectrum of activity has been further facilitated by the synthetic versatility of quinazolines which allows the generation of a large number of structurally diverse molecules.11 Taking into consideration the use of quinazoline derivatives in the treatment of some diseases, mentioned above, we have tested the antibacterial activity of the synthesized compounds 1 and 2 using strains of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Serratia marcescens stock cultures.

MATERIALS AND METHODS

General Experimental Procedure
All reagents and solvents were products of Sigma-Aldrich, Germany. Melting points were determined on a kofler hot stage apparatus and were uncorrected. IR spectra were recorded on a Buck scientific IR M500 instrument. The 1H- and 13C-NMR spectra were recorded in DMSO-d6 at 400 MHz with HAZ VOLATILE V2. M spectrophotometer. Chemical shifts were reported in ppm relative to tetramethylsilane. Gas chromatography-Mass spectra were obtained on a Finingan MAT 44S mass spectrometer operating at 70 eV. Elemental analysis agreed favourably with the calculated values. Analytical thin layer chromatography (TLC) was used to monitor the reactions.

Synthesis of 7-chloro-2-methyl-4H-benzo [d][1,3]-oxazin-4-one (1)
This involves the condensation of 0.76 g (0.005 mol) of 4-chloroantranilic acid with 1.02 g (10 mL, 0.01 mol) acetic anhydride in 30 mL ethanol medium. The reaction was heated under reflux with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (2 hours). (Yield = 2.01 g (96%), mp: 149-151°C).

Synthesis of 3-amino-7-chloro-2-methyl quinazolin-4(3H)one (2)
Equimolar amounts of 7-chloro-2-methyl-4H-benzo [d][1,3]-oxazin-4-one (1.61 g, 0.01 mol), and hydrazine hydrate (0.51 g, 0.01 mol) were heated under reflux in 30 mL ethanol with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (3 hours). (Yield = 1.50 g (95%), mp: 138-140°C). At the end of the reaction, the reaction mixture was concentrated in vacuo under reduced pressure using rotary evaporator. The white precipitate formed was then filtered, washed with distilled water (20 mL x 3). The white crystals were dried and recrystallized from dimethylformamide (DMF) to give pure 3-amino-7-chloro-2-methyl quinazolin-4(3H)-one.

Evaluation of Antibacterial Activity
Agar well diffusion method was utilized for the antibacterial activity.16 Six species: Staphylococcus aureus (ATCC10145), Bacillus species (NCTC
8236), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (NCTC 10418), *Serratia marcescens* (ATCC 14756) and *Pseudomonas aeruginosa* (ATCC 15442) stock cultures were used. The test organisms were obtained from the Pharmaceutical Microbiology Department of the University of Benin, Benin City, Nigeria. The test organisms were cultured overnight in nutrient broth, diluted to the turbidity of 0.5 McFarland standard. Broth culture (0.2 mL) were seeded on nutrient agar and allowed to dry. The various concentrations of the compounds (20 – 640 mg/mL) were introduced. The culture plates were incubated at 37°C for 24 h. The results were taken by considering the zones of inhibition by the test compounds. Ciprofloxacin (20 mg/mL) was used as positive control while the vehicle (10% DMSO) was used as negative control. Activity and inactivity were observed in accordance with standard and accepted method.

**Statistical Analysis**

Data were expressed as means ± SEM of triplicate determination. Student’s t-test was used to determine the significance of the difference between the control group and the test compounds.

**Results and Discussion**

The present study reported the synthesis of two derivatives of quinazolinone, 7-chloro-2-methyl-4H-benzo- [d][1,3] - oxazin-4-one (1) and 3-amino-7-chloro-2-methylquinazolin-4(3H)-one (2). The introduction of 2-amino substituent is a successful strategy to improve the chemical stability of benzoxazinone. Due to the pharmacological activities of 4(3H)-quinazolinone derivatives, 2,3-disubstituted derivative of quinazolin-4-one were synthesized via the interaction of the benzoxazinone derivative with nitrogen nucleophile with the aim of obtaining more precise information about the course of the reaction and some interesting pharmaceutical compounds. The reaction of 4, 5-disubstituted derivatives of methyl anthranilate and acetic anhydride yielded the cyclic compound 7-chloro-2-methyl-4H-benzo [d][1,3] - oxazin-4-one (Scheme 1). The reaction of this compound with hydrazine hydrate yielded 3-amino-7-chloro-2-methyl-quinazolin-4(3H)-one (Scheme 2).

The molecular formula of compound 1 was C_{10}H_{11}ClNO_{2} (m/z 195.602 [M]+). The IR spectrum showed signals for carbonyl functional group at 1662 cm^{-1}, C-O and C-H stretch vibrations at 1102 cm^{-1} and 2871 cm^{-1}, respectively. The 1H-NMR spectrum showed three aromatic protons at δH 7.59, 7.16 and 6.40 and a vinylic methyl protons at δH 2.57. In the 13C-NMR spectrum, the ester carbonyl resonated at δC 168.3, while the aromatic carbons resonated in the range δC 113.4 – 140.3. The resonance at δC 140.3 was due to the chlorinated carbon (C-5) while the resonances at δC 153.1, 149.2 and 22.1 were due to the carbons adjacent to the nitrogen of the oxazinone ring (C-1 and C-7) and the methyl carbon (C-9), respectively (Table 1).

Compound 2, molecular formula C_{12}H_{15}ClNO (m/z 209.633 [M]+), had NMR data similar to 1, except for an additional signal at δH 5.80 in the 1H-NMR spectrum which was attributed to the amino protons (2H) (Table 2).

**Table 1: 13C-NMR data of Compounds 1 and 2 (100 MHz in DMSO-d_{6}).**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>δC (Carbon atom number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>153.1 (C-2), 168.1 (C-4), 128.1 (C-5), 132.1 (C-6), 140.3 (C-7), 113.4 (C-8), 149.2 (C-9), 120.8 (C-10), 22.1 (C-11).</td>
</tr>
<tr>
<td>2</td>
<td>154.6 (C-2), 160.3 (C-4), 120.2 (C-10), 128.1 (C-5), 133.6 (C-6), 113.7 (C-8), 143.7 (C-7), 148.1 (C-9), 22.6 (C-11).</td>
</tr>
</tbody>
</table>

**Table 2: 1H-NMR of Compounds 1 and 2 (400 MHz in DMSO-d_{6}).**

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>δH (multiplicity, number of protons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.59 (s, 1H), 7.16 (s, 1H), 6.40 (s, 1H), 2.57 (s, 3H)</td>
</tr>
<tr>
<td>2</td>
<td>7.58 (s, 1H), 7.41 (s, 1H), 7.10 (s, 1H), 5.80 (s, 2H), 5.80 (s, 3H)</td>
</tr>
</tbody>
</table>

**Figure 1:** Effect of synthesized compounds (1 and 2) and positive control (ciprofloxacin) against test bacterial organisms. SA = *Staphylococcus aureus*, BS = *Bacillus species*, EC = *Escherichia coli*, KP = *Klebsiella pneumonia*, PA = *Pseudomonas aeruginosa*. Data represents mean ± SEM of triplicate determination. *Significant different from control (10% DMSO) at P < 0.05.

i = Acetic anhydride, ethanol

ii = Hydrazine hydrate, ethanol

**Scheme 1**

**Scheme 2**
Table 3: Minimum inhibitory concentrations (MIC) of compounds 1 and 2 against test bacterial organisms.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (mg/mL)</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>Escherichia coli</td>
<td>6.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus species</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>7.00</td>
<td>6.00</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>-</td>
<td>7.00</td>
<td>-</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>12.00</td>
<td>8.00</td>
<td>-</td>
</tr>
</tbody>
</table>

The compounds were investigated for their antimicrobial activity. The compounds synthesized exhibited promising antimicrobial activity against Staphylococcus aureus, Serratia marcescens, Escherichia coli and Klebsiella pneumonia. Both compounds were active against Staphylococcus aureus and Serratia marcescens. In addition, compound 1 showed activity against Escherichia coli while compound 2 was also active against Klebsiella pneumonia (Figure 1). Table 3 showed the MIC of both compounds against the susceptible organisms. Compound 2 had a slightly lower MIC (6 and 8 mg/mL) than compound 1 (7 and 12 mg/kg) against Staphylococcus aureus and Serratia marcescens, respectively (Table 3). This indicated that compound 2 is slightly more active against Staphylococcus aureus and Serratia marcescens compared to compound 1.

Conclusion
The present study has shown that the quinazolinone derivatives 1 and 2 have antibacterial activity with Compound 2 showing a higher activity against Staphylococcus aureus and Serratia marcescens compared to compound 1.

Conflict of interest
The authors declare no conflict of interest.

Authors’ declaration
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References