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# Synthesis and Antibacterial Activity of 7-chloro-2–methyl-4*H*-benzo[*d*][1,3]–oxazin-4-one and 3–amino-7-chloro-2-methyl quinazolin-4(3*H*)–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-4*H*-benzo[*d*][1,3]-oxazin-4-one (**1**) which further produced 3-amino-2-methyl-7-chloro quinazolin-4(3*H*)-one (**2**) via the reaction with hydrazine hydrate. The structures of the synthesized compounds were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance ($^{1}$H and $^{13}$C), Gas Chromatography-Mass Spectrophotometry and Elemental analysis. The synthesized compounds were screened for their antibacterial activity against various strains of bacteria: *Staphylococcus aureus*, *Bacillus species*, *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 antibacteria,[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 quinazolinone alkaloids, vasicinone 2 and deoxyvasicinone 3.[6]

In a quest to find additional quinazolinone-based potential drugs, various substituted quinazolinones 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 quinazolinone drug famous for its sedative hypnotic effects.[9]

The structural diversity of quinazolinones has been broadened with the discovery of asperlicin along with asperlicins B, C, D and E.[10-14] The broad spectrum of activity has been further facilitated by the synthetic versatility of quinazolinones which allows the generation of a large number of structurally diverse molecules.[15]

Taking into consideration the use of quinazolinone 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.

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## 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 $^{1}$H- and $^{13}$C-NMR spectra were recorded in DMSO-$d_6$ at 400 MH$_z$ 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 involve the condensation of 0.76 g (0.005 mol) of 4-chloroanthranilate 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$^{o}$C).

*Synthesis of 3-amino-7-chloro-2-methyl quinazolin-4-(3H)-one (**2**)*

Equimolar amounts of 7-chloro-2-methyl-4*H*-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$^{o}$C). At the end of the reaction, the reaction mixture was concentrated in vacuum 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(3*H*)-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 37ºC 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.[17]

*Statistical Analysis*

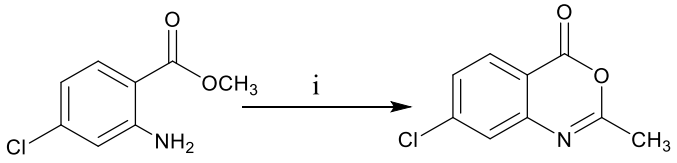
Data were expressed as means ± SEM of triplicate determination. Student's t-test was used to determine the significance of the difference between the control group and the test compounds.

## Results and Discussion

The present study reported the synthesis of two derivatives of quinazolinone, 7-chloro-2-methyl-4*H*-benzo-[*d*][1,3]-oxazin-4-one (**1**) and 3-amino-7-chloro-2-methylquinazolin-4-(3*H*)-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-4*H*-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-(3*H*)-one (Scheme 2).

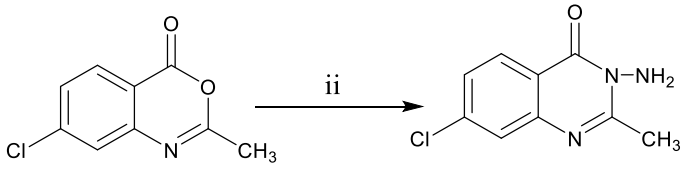
The molecular formula of compound **1** was $C_9H_6ClNO_2$ (*m/z* 195.602 [$M^+$]). The IR spectrum showed signals for carbonyl functional group at 1662 $cm^{-1}$, C-O and C-H stretch vibrations at 1102 $cm^{-1}$ and 2871 $cm^{-1}$, respectively. The $^1$H-NMR spectrum showed three aromatic protons at $\delta_H$ 7.59, 7.16 and 6.40 and a vinylic methyl protons at $\delta_H$ 2.57. In the $^{13}$C-NMR spectrum, the ester carbonyl resonated at $\delta_C$ 168.3, while the aromatic carbons resonated in the range $\delta_C$ 113.4 – 140.3. The resonance at $\delta_C$ 140.3 was due to the chlorinated carbon (C-5) while the resonances at $\delta_C$ 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 $C_9H_8ClN_3O$ (*m/z* 209.633 [$M^+$]), had NMR data similar to **1**, except for an additional signal at $\delta_H$ 5.80 in the $^1$H-NMR spectrum which was attributed to the amino protons (2H) (Table 2).




**Scheme 1**

i = Acetic anhydride, ethanol




**Scheme 2**

ii = Hydrazine hydrate, ethanol

**Table 1:** $^{13}$C-NMR data of Compounds 1 and 2 (100 MHz in DMSO-$d_6$).

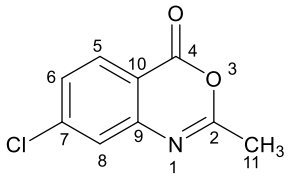
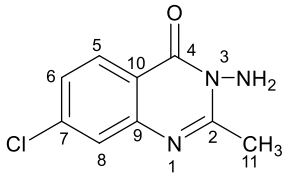
| Compound No. | $\delta_C$ (Carbon atom number) |
|---|---|
|  **1** | 153.1 (C-2), 168.1 (C-4), 128.1 (C-5), 132.1 (C-6), 140.3 (C-7), 113.4 (C-8), 149.2 (C-9), 120.8 (C-10) 22.1 (C-11). |
|  **2** | 154.6 (C-2), 160.3 (C-4), 120.2 (C-10), 128.1 (C-5), 133.6 (C-6), 113.7 (C-8), 143.7 (C-7), 148.1 (C-9), 22.6 (C- 11). |

**Table 2:** $^1$H-NMR of Compounds 1 and 2 (400 MHz in DMSO-$d_6$).

| Compound No. | $\delta_H$ (multiplicity, number of protons) |
|---|---|
| **1** | 7.59 (s, 1H), 7.16 (s, 1H), 6.40 (s, 1H), 2.57 (s, 3H) |
| **2** | 7.58 (s, 1H),7.41 (s, 1H), 7.10 (s, 1H), 5.80 (s, 2H), 2.58 (s, 3H) |

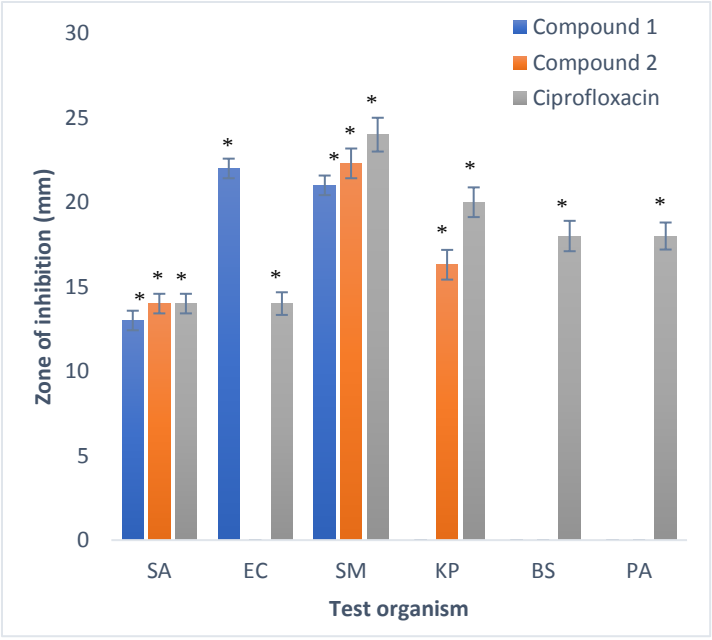



**Figure 1:** Effect of synthesized compounds (1 and 2) and positive control (ciprofloxacin) against test bacterial organisms. SA = *Staphylococcus aureus*, BS = *Bacillus species*, EC = *Escherichia coli*, KP = *Klebsiella pneumonia*, PA = *Pseudomonas aeruginosa*. Data represents mean ± SEM of triplicate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

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

| Test organism | MIC (mg/mL) | |
|---|---|---|
| | **1** | **2** |
| *Escherichia coli* | 6.00 | - |
| *Bascillus species* | - | - |
| *Staphylococcus aureus* | 7.00 | 6.00 |
| *Klebsiella pneumonia* | | 7.00 |
| *Serratia marcescens* | 12.00 | 8.00 |

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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