Synthesis and antibacterial activity of newly synthesized 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazin-4-one and 3-amino-7-chloro-2-methyl-quinazolin-4(3H)-one

Osarumwense Peter Osarodion *

Department of Chemical Science, Ondo State University of Science and Technology, Okitipupa Ondo State, Nigeria.

Publication history: Received on 21 April 2020; revised on 29 April 2020; accepted on 29 April 2020

Abstract

The current study is aimed at the synthesis of these quinazolinone derivatives 7-Chloro-2-Methyl-4H-benzo[d]-[1,3]-Oxazin-4-one and 3-Amino-7-Chloro-2—Methyl-3H-Quinazolin-4-One and evaluate them for their antibacterial activity. The condensation of 2-amino-methyl-4-methoxybenzoate with acetic anhydride yielded the cyclic compound 2-methyl-4,5-disubstituted-1,3-benzo-oxazine-4-one which further produce a novel 2,3-disubstituted quinazolin-4 ones via the reaction with hydrazine hydrate. The quinazolinone derivatives 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazin-4-one and 3-amino-7-chloro-2-methyl-quinazolin-4(3H)-one were evaluated pharmacologically for their in vivo analgesic activities by acetic acid induced writhing in mice. The compounds synthesized were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance (1H and 13C), Gas Chromatography Mass Spectrophotometer and Elemental analysis. The synthesized compounds were screened against various strains of microorganism; Klebsiella pneumonia, Staphylococcus aureus, Bacillus species, Escherichia coli, Klebsiella pneumonia, and Candida albicans. Compounds 1 and 2 showed significant activity against Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa with MIC ranging from 6 – 9 mg/mL. The test investigated compounds exhibited significant antibacterial activity against the bacteria when compared with the control test sample. The IR spectra of compound 1 were characterized by absence of υ NH2 and presence of υ C=O stretch in 1157 cm⁻¹ region of the compound. Compound 2 was characterized by absence of υ C=O and presence of υNH2 in 3285 cm⁻¹ and 3184 cm⁻¹ region of the compound. The compounds synthesized exhibited promising antibacterial activities against Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa, stock cultures. The compounds have high activity against the microorganisms. Compound 2 has a higher activity against Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus compared to Compound 1.

Keywords: 3-amino-7-chloro-2-methyl-quinazolin-4(3H)-one; 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazine-4-one; quinazolin-4(3H)-one; antibacterial activity; Nucleophile, Synthesis; Reaction mixture; Klebsiella pneumonia; Staphylococcus aureus

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| TLC          | Thin Layer Chromatography |
| SEM          | Standard error mean |
| IR           | Infrared Spectra |
| UV/Visible   | UV-Visible Spectra |

*Corresponding author: Osarumwense Peter Osarodion

Copyright © 2020 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.
1. Introduction

Quinazolinone derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity. They are widely used in pharmaceuticals and agrochemicals. Several reports have been published on the biological activities of quinazolinone derivatives, including their anti-inflammatory [1–7], antimalarial [8, 9], antimicrobial, anti-fungal, antibacterial [10–16], anticonvulsant [17–20], and antitumor [21, 22].

Quinazolinone derivatives with 2, 3- substitution are reported to possess significant analgesic and anti-inflammatory activity [23, 24]. Looking at the biological significance of quinazolinone nucleus, it was thought to synthesize new quinazolinone derivatives and screen them for their analgesic activity.

One of the medicinally important heterocyclic compounds is the quinazoline. Quinazoline is a compound made up of two fused six-membered simple aromatic rings, benzene ring and a pyrimidine ring. Quinazoline, earlier known as benzo-1, 3-diazine was first prepared in the laboratory by Gabriel in 1903, although one of its derivatives was known much earlier [25].

The name quinazoline (German; Chinazolin) was first proposed for this compound by Weddige, on observing that this was isomeric with the compounds Cinnolin and Quinoxaline. Paal and Bush suggested the numbering of quinazoline ring system, which is currently used. The other less commonly used names for this ring system are phemiazine and 5,6-benzopyrimidine. However, the name quinazoline is now universally accepted [26].

There are four isomers of Quinazoline which are identified by nitrogen positions;

Cinnoline [1,2 – Benzodiazine],

\[ \text{Cinnoline} \]

Phthalazine [2,3- Benzodiazine],

\[ \text{Phthalazine} \]

Quinazoline [1,3-Benzodiazine],

\[ \text{Quinazoline} \]
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, Pseudomonas aeruginosa, Bacillus species, Escherichia coli, Klebsiella pneumonia, and Candida albicans stock cultures.

2. Material and methods

2.1. General experimental procedure

Reagents and solvents were purchased from sigma-Aldrich chemical supplier in Germany. Melting points were determined on a Kofler hot stage apparatus and are 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 400MHz, with HAZ VOLATILE V2.M. Chemical shifts are reported in ppm relative to tetramethylsilane period. Gas chromatography mass (GC/MS) spectra were obtained on a Finingan MAT 44S mass spectrometer operating at electron impact energy of 70eV. Elemental analysis data agreed with the calculated values. Analytical thin layer Chromatography (TLC) was used to monitor the reactions.

2.1.1. Synthesis of 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazine-4- one, quinazolin-4(3H)-one, (1)

This involved the condensation of Methyl-2-amino-5-methoxyl-benzoate "$2.11g$ (0.01mol) and $1.02g$ (0.01mol) acetic anhydride in 30ml ethanol medium were reacted. 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). At the end of the reaction, work up was done. Ethanol was removed in vacuum and the crude mixture was poured into 50ml of ice water on a cold water bath. The mixture was stirred for 30 minutes filtered and extracted into ethyl acetate and allowed to evaporate at room temperature to give solid products which were recrystallized from hexane or dichloromethane-hexane mixture. Yield was "$2.01g$ (95%), mp: "$148-150^\circ$C.

2.1.2. Synthesis of 3-amino-7-Chloro 2-Methyl quinazoline-4-(3H)-One. (2)

The condensation of equimolar amounts of 2-methyl, 6-methoxyl-4H-benzo [D] [1, 3]–oxazine-4-one "$1.06g$, ("0.005" mol) and hydrazine hydrate "$0.93g", ("0.01" mol) were added to $30ml$ boiling ethanol with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (3 hours).

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 three times with $20ml$ of distilled water [20ml x 3]. The white crystals were dried and recrystallized from dimethylformamide (DMF) to give pure 3-amino-7-Chloro 2-Methyl quinazoline-4-(3H)-one. Yield was "$1.00g$ (94%) mp: "$97-99^\circ$C.

2.2. Evaluation of antimicrobial activity

Agar well diffusion method was utilized for the antimicrobial activity [27]. Six species: Staphylococcus aureus (ATCC10145), Bacillus species (NCTC 8236), Escherichia coli (ATCC 25923), Klebsiella pneumonia (NCTC 10418), Serratia marcescens (ATCC 14756) and Candida albicans (ATCC24433) 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 (for bacterial organisms) or Sabouraud dextrose agar (for the fungus) 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 (for bacterial organisms) or at room temperature (28°C) for 48 h (for the fungus). 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 [28].

![Possible Mechanism](image)

**Figure 1** Where: $R_1 = H$, $R_2 = H$, and $R_3 = Cl$

![Scheme 1](image)

$i =$ Acetic anhydride, ethanol

**Figure 2 Where:** $R_1 = H$, $R_2 = H$, and $R_3 = Cl$

![Scheme 2](image)

$ii =$ Hydrazine Hydrate, ethanol
Possible Mechanism

2.3. Elemental analysis

The compositions of the compounds are summarized in table 1. The C and H contents (both theoretically calculated values and actual values) are indicated.

3. Results

3.1. Antibacterial activity of control drugs and tested compounds against tested standard organism

3.1.1. Control Drugs

Ciprofloxacin (CPX) For Bacteria
Ketonaxol (PEF) For Fungus

Compound 1 (1)
Compound 2 (2)

Figure 3 The effect of Compounds toward studied bacteria. SA=Staphylococcus aureus, BS=Bacillus species, EC=Escherichia coli, KP=Klebsiella pneumonia, SM=Serratia marcescens and CA=Candida albicans

Significantly different from Ligand at P< 0.05, values are in mm
3.2. Characterization of 7-Chloro-2-Methyl-4H–benzo[d] [1,3]–oxazine–4–one (1)

$^1$H NMR (400MHz, DMSO) δ 7.49 (s, 1H), 7.14 (s, 1H), 6.30 (s, 1H), 2.53 (s, 3H). $^{13}$C NMR (400MHz, DMSO) δ 168.05, 140.10, 149.40, 153.07, 141.33, 134.40, 127.03, 114.40, 23.42. IR (KBr, cm$^{-1}$) 3381, 3203, 3135, (NHz), 3018 (CH aromatic), 2951, 2871, 2718 (CH aliphatic), 1662 (C=O). Anal. Cal. 1159 (C=O) for C$_9$H$_6$ClN$_2$O; C 61.10; H 4.13. Found: C 61.20, H 4.76.

3.3. Characterization of 3-amino-7-Chloro 2-Methyl quinazoline-4-(3H)-One (2)

$^1$H NMR (400 MHz, DMSO) δ 7.48 (s, 1H), 7.31 (s, 1H), 7.04 (s, 1H), 5.79 (s, 2H), 2.56 (s, 3H) $^{13}$C NMR (400MHz, DMSO) δ 161.20, 142.50, 154.10, 148.20, 134.60, 121.30, 114.10, 127.60, 148.20, 23.10. IR (KBr, cm$^{-1}$) 3301 (NH$_2$), 1622 (C=O), Anal. Cal. for C$_9$H$_8$N$_3$O; C 54.21, H 4.43; Found, C 54.30, H 4.31.

Table 1 Characterization and physical data of synthesized compounds

| Compound No | Solvent | Formula M. wt | Analysis% Calc/Found |
|-------------|---------|---------------|----------------------|
| 1           | Ethanol | C$_9$H$_6$NCl$_2$O$_2$ (195.602) | 61.10/4.13 |
| 2           | Ethanol | C$_9$H$_8$N$_3$Cl$_2$O (209.633) | 54.21/4.43 |

Table 2 $^{13}$C-NMR of synthesized compounds

| Compound No | δ (ppm) Carbon atom number |
|-------------|----------------------------|
| 1           | 168.05(C-2), 140.10(C-6), 149.40(C-8) |
|             | 153.07(C-1), 141.33 (C-5), 134.40 (C-4) |
|             | 127.03 (C-3), 114.40 (C-7), 23.42(C-9). |
| 2           | 161.20(C-2), 142.50 (C-6), 154.10 (C-1) |
|             | 148.20 (C-8), 134.60 (C-5), 121.30 (C-3), 114.10 (C-7), 127.60 (C-4), 148.20(C-8), 23.10 (C-9) |
Table 3 $^{13}$C-NMR of synthesized compounds

| Compound No | $\delta$ (ppm) |
|-------------|----------------|
| ![Compound 1](https://example.com/compound1.png) | 7.49 (s, 1H), 7.14 (s, 1H), 6.30 (s, 1H), 2.53 (s, 3H) |
| ![Compound 2](https://example.com/compound2.png) | 7.48 (s, 1H), 7.31 (s, 1H), 7.04 (s, 1H), 5.79 (s, 2H), 2.56 (s, 3H) |

Table 4 Minimum inhibitory concentrations (MIC) in mg/mL of tested compounds against tested standard microorganisms

| TEST ORGANISM       | COMPOUND | 1   | 2   |
|---------------------|----------|-----|-----|
| Escherichia coli    |          | 6.00| -   |
| Klebsiella pneumonia|          | -   | 7.00|
| Staphylococcus aureus|      | 6.00| 6.00|
| Pseudomonas Aeuriginosa|   | 9.00| 8.00|
| Bacillus species    |          | -   | -   |
| Candida albicans    |          | -   | -   |

4. Discussion

The present study reported the synthesis of two derivatives of quinazolinone, 3-amino-7-chloro-2-methyl-quinazolin-4(3H)-one (1) 7-chloro-2-methyl-4H-benzo[d][1,3]-oxazine-4-one, quinazolin-4(3H)-one, (2). The compounds were investigated for their Antimicrobial activity. Structural elucidations of compounds synthesized were characterized by correct elemental analysis and careful inspections of spectral data. Looking at the $^1$H NMR spectra of the compounds synthesized, compound 1 displayed a singlet at $\delta$ 2.53 which was due to methyl group. Other singlets appeared at $\delta$ 7.49 and 7.14 attributed to aromatic protons. Also, $^1$H NMR spectrum of compound 2 showed a characteristic signal at $\delta$ 2.56 (singlet) corresponding to methyl group. Two singlets appeared at $\delta$ 7.48 and 7.31 attributed to aromatic protons. Another signal appeared at 5.79 which was attributed to the protons of the amino group. For the IR spectra, compound 1 were characterized by absence of $\nu$ NH$_2$ and presence of $\nu$ C-O stretch in 1662cm$^{-1}$ region of the compound. Compound 2 was characterized by absence of $\nu$ C-O and presence of $\nu$NH$_2$ in 3301cm$^{-1}$ region of the compound.

The $^{13}$C NMR spectrum of compound 1, revealed signals at $\delta$23.40, attributed to methyl group, while the aromatic carbon atoms appeared between $\delta$ values 114.40-168.50 with the carbonyl carbon atom appearing as the highest $\delta$ value of 168.50. Similarly, compound 2 showed signals at 823.10, attributed to methyl group, while the aromatic carbon atoms appeared between $\delta$ values 114.10 – 161.20, with the carbonyl carbon atom appearing as the highest $\delta$ value of 161.20. The compounds synthesized exhibited promising antimicrobial activities against Klebsiella pneumonia, Staphylococcus aureus, and Pseudomonas aeuriginosa stock cultures.
5. Conclusion
The present study has showed that the quinazolinone derivatives 1 and 2 have antibacterial activity. Compound 2 has a higher activity against Klebsiella pneumonia, Staphylococcus aureus, and Pseudomonas aeruginosa, compared to Compound 1.

Compliance with ethical standards

Acknowledgments
The author acknowledge the assistance of Baba Haruna of the Department of Pharmaceutical Chemistry of Niger Delta University, Wilberforce Island, Yenogoa and Dr. Marris, in England for running the spectra.

Disclosure of conflict of interest
The author declares no conflict of interest.

References
[1] A Kumar, S Sharma, Archana et al. (2003). Some new 2,3,6-trisubstitutedquinazolinones as potent anti-inflammatory, analgesic and COX-II inhibitors. Bioorganic and Medicinal Chemistry, 11(23), 5293–5299.
[2] B Maggio, G Daidone, D Raffa et al. (2001). Synthesis and pharmacological study of ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1H-pyrazole-4-acetates. European Journal of Medicinal Chemistry, 36(9), 737–742.
[3] RS Giri, HM Waker, T Giordano et al. (2009). Design, synthesis and characterization of novel 2-(2,4-disubstituted-thiazole-5-y1)-3aryl-3H-quinazoline-4-one derivatives as inhibitors of NF-kB and AP-1 mediated transcription activation and as potential anti-inflammatory agents. European Journal of Medicinal Chemistry, 44(5), 2184–2189.
[4] E Manivannan and SC Chaturvedi. (2011). Analogue-based design, synthesis and molecular docking analysis of 2,3-diaryl quinazolinones as non- ulcerogenic anti-inflammatory agents. Bioorganic and Medicinal Chemistry, 19(15), 4520–4528.
[5] A Kumar, CS Rajput and SK Bhati. (2007). Synthesis of 3-[4′-(p-chlorophenyl)-thiazol-2′-yl]-2-[(substituted azetidinone/ thiazolidinone)-aminomethyl]-6-bromoquinazolin-4-ones as anti-inflammatory agent. Bioorganic and Medicinal Chemistry, 15(8), 3089–3096.
[6] RS Giri, HM aker, T Giordano, et al. (2010). Design, synthesis and evaluation of novel 2-thiophen-5-yl-3Hquinazolin-4-one analogues as inhibitors of transcription factors NF-kB and AP1 mediated transcriptional activation: their possible utilization as anti-inflammatory and anti-cancer agents. Bioorganic and Medicinal Chemistry, 18(7), 2796–2808.
[7] E Bansal, V K Srivastava and A Kumar. (2001). Synthesis and anti-inflammatory activity of 1-acetyl-5-substitute daryl-3-(ββ-aminophenyl)-2-pyrazolines and ββ-(substitute daminoethyl) amidonaphthalenes. European Journal of Medicinal Chemistry, 36(1), 81–92.
[8] SZhu, J Wang, G Chandrashekar, E Smith, X Liu and Y Zhang. (2010). Synthesis and evaluation of 4-quinazolinones compounds as potential antimalarial agents. European Journal of Medicinal Chemistry, 45(9), 3864–3869.
[9] S Zhu, Q Zhang, CGudise, L Wei, E Smith and Y Zeng. (2009). Synthesis and biological evaluation of febrifugine analogues as potential antimalarial agents. Bioorganic and Medicinal Chemistry, 17(13), 4496–4502.
[10] GP Suresha, R Suhas, W Kapfo and D Channe Gowda. (2011). Urea/thiourea derivatives of quinazolinone-lysine conjugates: synthesis and structure-activity relationships of a new series of antimicrobials. European Journal of Medicinal Chemistry, 46(6), 2530–2540.
[11] MS Mohameda, MM Kamel, EM Kassem, N Abotaleb, SI AbdEl-Moez and MF Ahmed. (2010). Novel 6,8-dibromo-4(3H)quinazolinone derivatives of anti-bacterial and antifungal activities. European Journal of Medicinal Chemistry, 45(8), 3311–3319.
[12] DR Patel and KC Patel. (2011). Synthesis, antimicrobial activity and application of some novel quinazolinone based monoazo reactive dyes on various fibres. Dyes and Pigments, 90(1), 1–10.
[13] D Kohli, SR Hashim, S Vishal, M Sharma and AK Simgh. (2009). Synthesis and antibacterial activity of quinazolinone derivatives. International Journal of Pharmacy and Pharmaceutical Sciences, 1(1), 163–169.

[14] NB Patel and JC Patel. (2011). Synthesis and antimicrobial activity of Schiff bases and 2-azetidinones derived from quinazolin 4(3H)-one. Arabian Journal of Chemistry, 4(4), 403–411.

[15] SN Pandeya, DSriram, GNath and E De Clercq. (1999). Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino2-methylmercapto quinazolin-4(3H)-one. Pharmaceutica Acta Helvetiae, 74(1), 11–17.

[16] A Kumar, P Sharma, P Kumari and BLalKalal. (2011). Exploration of antimicrobial and antioxidant potential of newly synthesized 2, 3-disubstituted quinazoline-4(3H)-ones. Bioorganic and Medicinal Chemistry Letters, 21(14), 4353–4357.

[17] MZappala, S Grasso, NMicale et al. (2003). 1-Aryl-6, 7- methylenedioxy-3H-quinazolin-4-ones as anticonvulsant agents. Bioorganic and Medicinal Chemistry Letters, 13(24), 4427–4430.

[18] V Jatav, PMishra, SKashaw and JP Stables. (2008). CNS depressant and anticonvulsant activities of some novel 3-[5- substituted 1, 3, 4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H) - ones. European Journal of Medicinal Chemistry, 43(9), 1945–1954.

[19] AS El-Azab and KEHEITahir. (2012). Synthesis and anticonvulsant evaluation of some new 2, 3, 8-trisubstituted-4(3H)-quinazoline derivatives. Bioorganic & Medicinal Chemistry Letters, 22(1), 327–333.

[20] SK Kashaw, VKashaw, PMishra, NK Jain and JP Stables. (2009). Synthesis, anticonvulsant and CNS depressant activity of some new bioactive 1-(4-substituted-phenyl)-3-(4-o xo-2- phenyl/ethyl-4H-quinazolin-3-yl)-urea. European Journal of Medicinal Chemistry, 44(11), 4335–4343.

[21] SL Cao, YP Feng, YY Jiang, SY Liu, GY Ding and RT Li. (2005). Synthesis and in vitro antitumor activity of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains. Bioorganic and Medicinal Chemistry Letters, 15(7), 1915–1917.

[22] AM Al-Obaid, SG Abdel-Hamide, HA El-Kashef et al. (2009). Substituted quinazolines, part 3. Synthesis, in vitro antitumor activity and molecular modeling study of certain 2-thieno 4(3H)-quinazolinone analogs. European Journal of Medicinal Chemistry.

[23] AE Abdel- Rahman, EA Bakhte and EA Al- Taifi. (2003). Synthesis and anti-microbial testing of some new S. Substituted thiopyridines, thienopyridines, pyridothenopyrimidines and pyridothenotriazines. Pharmazine, 58, 372-377.

[24] RVChambhare, BG Khadse, AS Bobde and RH Bahekar. (2003). Synthesis and preliminary evaluation of some N- [5-(2- furanyl)-2-methyl-4-oxo-4H-thieno [2, 3-d] pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3H- thieno [2, 3-d] pyrimidin-4-ones as anti-microbial agents. Eur. J. Med. Chem, 38, 89-100

[25] Wikipedia. (2011). Organic chemistry.

[26] Meyyanathan SN. (2005). Chemistry of quinazolinones. Pharmainfo.net, 3(3).

[27] Okeke ML, Iroegbu CU, Eze EN, Okoli AS and Esimone CO. (2001) Evaluation of extracts of the root of Landolphiaowerrienasefor antibacterial activity. J. Ethnopharmacol, 78, 119 - 127.

[28] Mackie R and Cartney MC. (1984). Practical Medicinal Microbiology 3rd edition, Vol.2 Churchill Livingstone (Publishers), London and New York, 121(141), 100 – 106.

How to cite this article
Osarumwense PO. (2020). Synthesis and antibacterial activity of newly synthesized 7-chloro-2-methyl-4h-benzo[d] 1, 3-oxazin-4-one and 3-amino-7-chloro-2-methyl-quinazolin-4(3h)-one. GSC Biological and Pharmaceutical Sciences, 11(1), 212-220.