Synthesis and antibacterial activity of 3-amino-6-iodo-2-methyl quinazolin 4-(3H)-one and 6-iodo-2-methyl-4H-benzo [D] [1, 3] oxazin-4-one

Osarumwense Osarodion Peter *

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

Abstract

Quinazolines and its derivatives represent one of the most active classes of compounds, which possess wide range of biological activities like anti-bacterial, analgesic, anti-microbial, anti-inflammatory, anticancer, and anti-hypertensive, antifungal, anti-HIV, antioxidant, analgesic, anticonvulsant, antimalarial, antitumor, anti-tubercular activities. The objective of the present study was to synthesize these quinazolinone derivatives 6-iodo-2-methyl-4H-benzo[d]-[1,3]-oxazin-4-one and 3-amino-6-iodo-2—methyl-3H-quinazolin-4-one and evaluate them for their antibacterial activity.

6-iodo-4H-benzoxazin-4-one was synthesized by the reaction of 2-amino-5-Iodomethylbenzoic acid and acetic anhydride which reacted with nitrogen nucleophile, namely hydrazine hydrate to obtain 3-amino-6-iodo-2-methyl-3H-quinazolin-4-one. The structures of the compounds were confirmed with Infrared Spectra, Proton Nuclear Magnetic Resonance, Carbon thirteen Nuclear Magnetic Resonance, mass spectra and elemental analysis. These compounds were screen for their antibacterial activities against a number of microorganisms, Escherichia coli, Klebsiella pneumonia, Bacillus species, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albican. The test investigated compounds exhibited significant antibacterial activity against the bacteria when compared with the control test sample. For the IR spectra, compound 1 were characterized by absence of $\nu$ NH$_2$ and presence of $\nu$ C=O stretch in 1159 cm$^{-1}$ region of the compound. Compound 2 was characterized by absence of $\nu$ C-O and presence of $\nu$NH$_2$ in 3284 cm$^{-1}$and 3194 cm$^{-1}$ region of the compound. The compounds synthesized exhibited promising antibacterial activities against Staphylococcus aureus, Bacillus species and Pseudomonas aeruginosa, stock cultures. The compounds have high activity against the microorganisms. Compound 2 has a higher activity against Pseudomonas aeruginosa compared to compound 1.

Keywords: Antibacterial activity; Synthesis; Acetic anhydride; Hydrazine hydrate; Nucleophile; Quinazolin-4(3H)-one

1. Introduction

Having witnessed from literature, the enormous importance of quinazolinones [1 - 8]. Promising antimicrobial and antifungal activities have been reported in many substituted quinazoline derivatives. Earlier, Quinazoline-4-ones has been a subject of extensive pharmacological evaluation, as well as, toxicological studies for antimicrobial and antifungal activities [9]. A brief survey on the biological activities of quinazolin- 4 (3H) – one derivatives showed anti-inflammatory [10, 11], antitumor [12 – 15] anti HIV [16-17], antibacterial [18 - 19]. As well as CNS depressant and anticonvulsant activities [20 – 21].

Microbial infections cause pain and inflammation in the body. Generally two groups of agents are given for normal practice simultaneously (anti-microbial, analgesic and antiinflammatory effect). Compound with all three properties are not very common. The commercially available antimicrobial agents are having many adverse effects [22]. In view of the associated biological and pharmacological properties of heterocycles. We planned to screen these derivatives of quinazolin-4-one for their possible antibacterial activity [23].

* Corresponding author
E-mail address: osarodion.peter@yahoo.com

Copyright © 2019 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.
2. Material and methods

General experimental procedure

All reagents and solvents were purchased from sigma-Aldrich, in Germany. Melting points were determined on a kofler hot stage apparatus and were uncorrected. IR spectra were recorded on a Buck scientific IR M500.

The $^1$H and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ at 400 MHz with HAZ VOLATILE V2. M Chemical shifts are reported in ppm relative to tetramethylsilane. Gas chromatography mass spectra were obtained on a Finingan MAT 44S mass spectrophotometer operating at 70eV. Elemental analysis was carried out with analytical Thin layer chromatography (TLC) was used to monitor the reactions.

2.1. 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.

![Figure 1](image1.png)

**Figure 1** possible mechanism for synthesis of compound 1

![Figure 2](image2.png)

**Figure 2** possible mechanism for synthesis of compound 2

2.2. Procedure for the synthesis of 6-iodo-2-methyl-4h-benzo[d]-[1,3]-oxazin-4-one(1).

Benzoazininone has been synthesized by following the procedure of Bogert and Seil. A mixture of 1 (0.01 mole, 2.77 g) and acetic anhydride (0.02 mole, 1.02 g) was refluxed for 1-2h. The mixture was cooled, evaporated and the residue was washed with water and re-crystallized from ethanol to afford compound 2. Yield (75%), m.p 154-155°C, IR (v/cm$^{-1}$): 3012(C-H aromatic), 2925 (C-H aliphatic), 1760 (C=O), 1620(C=N), MS: m/z 287 (M$^+$). $^1$HNMR (CDCl$_3$): $\delta$ =7.32 - 7.10 (m, 3H, ArH), 1.61 (s, 3H, CH$_3$). Anal. Calc. for C$_9$H$_6$N$_0$2I s: C: 37.63, H, 2.09; N, 4.88%. Found: C, 37.78, H, 2.39, N, 5.09.
2.3. Procedure for the synthesis of 3-amino-6-iodo-2—methyl-3H-quinazolin-4-one (2).

A mixture of benzoxazinone (2) (2.87g, 0.01mole) and hydrazine hydrate (1g, 0.02mole) was heated under reflux in absolute ethanol (30 ml) for 3h. The reaction mixture was concentrated. After cooling, the solid was separated out, filtered off, dried, and then re-crystallized from ethanol to afford quinazolinone (3). Yield (80%, m. p. 158°C: IR (v/cm−1): 3284, 3194, (NH2), 3046 (C-H aromatic), 1660 (C=O), 1596 (C=N) MS: m/z 301 (M+), Anal. Calc. for C9H6IN3O: C. 35.90: H, 2.68; I 42.15, N.13.96.

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 quinazoline-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 2-amino-5-iodomethylbenzoat and acetic anhydride yielded the cyclic compound 6-iodo-2-methyl-4H-benzo[d][1,3]-oxazin-4-one. The reaction of this compound with hydrazine hydrate yielded 3-amino-6-iodo-2-methyl-3H-quinazolin-4-one.

2.4. Evaluation of antimicrobial activity

Agar well diffusion method was utilized for the antimicrobial activity [24]. Six species: Staphylococcus aureus (ATCC10145), Bacillus species (NCTC 8236), Escherichia coli (ATCC 25922), Klebsiella pneumonia (NCTC 10418), Pseudomonas aeruginosa (ATCC 15692) 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) 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% Dimethysulphuside was used as negative control. The results were assessed by measuring the zone of growth inhibition by the test compound. [25]. Activity and inactivity were observed in accordance with standard and accepted method.

2.5. Statistical analysis

All data were expressed as means ± SEM; the student’s t-test was applied to determine the significance of the difference between the control group and the test compounds.

3. Results

The effect of compounds toward studied bacteria. SA = Staphylococcus aureus, BS = Bacillus species, EC = Escherichia coli, KP = Klebsiella pneumonia, PA = Pseudomonas aeruginosa and CA = Candida albicans. Significantly different from Ligand at P< 0.05, values are in mm
Figure 4 antibacterial activity of compound 1 against tested standard organism

The effect of compounds toward studied bacteria. SA = Staphylococcus aureus, BS = Bacillus species, EC = Escherichia coli, KP = Klebsiella pneumonia, PA = Pseudomonas aeruginosa and CA = Candida albicans. Significantly different from Ligand at P< 0.05, values are in mm

Figure 5 antibacterial activity of compound 2 against tested standard organism

The effect of compounds toward studied bacteria. SA = Staphylococcus aureus, BS = Bacillus species, EC = Escherichia coli, KP = Klebsiella pneumonia, PA = Pseudomonas aeruginosa and CA = Candida albicans. Significantly different from Ligand at P< 0.05, values are in mm

3.1. 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.

Table 1 characterization and physical data of synthesized compounds

| Compound No | Solvent | Formula (Molecular weight) | Analysis % | C and H contents |
|-------------|---------|----------------------------|------------|------------------|
| 2           | Ethanol | C9H6N2O2I (287)            | 37.81      | 2.05             |
|             |         |                            | 37.63      | 2.09             |
| 3           | Ethanol | C9H8N3O (301)              | 35.90      | 2.40             |
|             |         |                            | 35.88      | 2.66             |

Table 2 13C-NMR of synthesized compounds

| Compound No | δ (ppm) Carbon atom number |
|-------------|----------------------------|
| 1           | 168.28(C-2), 156.10(C-1), 149.23(C-8), 140.28 (C-4), 113.37 (C-5), 100.56 (C-6) 100.05 (C-3), 100.01 (C-7), 16.95 (C-9) |
| 2           | 160.28 (C-2), 156.21(C-1), 154.57 (C-8), 149.07 (C-4), 143.77 (C-5), 113.65 (C-6) 108.24 (C-3), 105.64 (C-7), 22.58 (C-9). |
Table 3 1H-NMR of synthesized compounds

| Compound No | δ (ppm) |
|-------------|---------|
| 1           | 7.32 – 7.10 (m, 3H, ArH), 1.16 (s, 3H, CH3). |
| 2           | 7.52 – 7.15 (m, 3H, ArH), 5.80 (s, 2H), 2.58 (s, 3H) |

Table 4 minimum inhibitory concentrations (MIC) in mg/ml of sample compounds against tested standard microorganisms

| Test organism                 | Compound |
|-------------------------------|----------|
| Escherichia coli              | 6.00     |
| Klebsiella pneumonia          | -        |
| Staphylococcus aureus         | 7.00     |
| Pseudomonas aureus            | 10.00    |
| Bacillus cereus               | -        |
| Candida albicans              | -        |

3.2. Characterization of 7-iodo 2-methyl-4h-benzo [d][1,3] -oxazin-4-one (1).

1H NMR (400MHz, DMSO) δ 7.32 – 7.10 (s, 3H, ArH ), 1.61 (s, 3H,CH3), 13C NMR (400MHz, DMSO) δ 168.28, 156.10, 149.23, 140.28, 113.37,100.56, 100.05, 100.01, 16.95. IR (KBr, cm⁻¹)3135, (NH₂), 3012 (CH aromatic), 2925, 2871, 2718 (CH aliphatic), 1760 (C=O), 1620 (C=N), Anal.Cal 1159 (C=O) for C₉H₆N₀I; C 55.21; H 3.07. Found: C 55.22, H 3.08.

3.3. Characterization of 3-amino-7-iodo-2-methyl-quinazoline-4(3H)-one (2).

1H NMR (400 MHz, DMSO) δ 7.52 – 7.15 (m, 3H, ArH), 5.80 (s, 2H), 2.58 (s, 3H), 13C NMR (400MHz, DMSO) δ 160.28, 156.21,154.57, 149.07, 143.77, 113.65, 108.24, 105.64, 22.58, IR (KBr,cm⁻¹) 3284,3194(NH₂),3046(C=H aromatic), 1660 (C=0),1596(C-N), Anal. Cal.for C₉H₈IN₃O; C 51.52; H 3.82; Found, C 51.53, H 3.83.

4. Discussion

The present study reported the synthesis of two derivatives of quinazolinone, 7-iodo-2-methyl-4h-benzo [d] [1,3]-oxazin-4-one (1) and 3-amino-7-iodo-2-methyl 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 1H NMR spectra of the compounds synthesized, compound 1 displayed a singlet at δ 1.61 which was due to methyl group. Other singlets appeared at δ7.32 and 7.10 attributed to aromatic protons. Also, 1H NMR spectrum of compound 2 showed a characteristic signal at δ 2.58 (singlet) corresponding to methyl group. Multiplets appeared at δ7.52 – 7.15 attributed to aromatic protons. Another signal appeared at 5.80 which was attributed to the protons of the amino group. For the IR spectra, compound 1 were characterized by absence of υ NH₂ and presence of υ C=O stretch in 1159cm⁻¹ region of the compound. Compound 2 was characterized by absence of υ C=O and presence of υ NH₂ in 3284cm⁻¹ and 3194cm⁻¹ region of the compound.

The 13C NMR spectrum of compound 1, revealed signals at δ16.95, attributed to methyl group, while the aromatic carbon atoms appeared between δ values 100.05-168.28 with the carbonyl carbon atoms appearing as the highest δ value of 168.28. Similarly, compound 2 showed signals at δ22.58, attributed to methyl group, while the aromatic carbon atoms appeared between δ values 105.64-160.28, with the carbonyl carbon atom appearing as the highest δ value of 160.28. The compounds synthesized exhibited promising antibacterial activities against Staphylococcus aureus, Bacillus species and Pseudomonas aeruginosa, stock cultures.
5. Conclusion

The compounds have high activity against the microorganisms. Compound 2 has a higher activity against Pseudomonas aeruginosa compared to Compound 1. These compounds synthesized could be a potential antibiotic and a tool in Pharmaceutical drug delivery. Clinical trials need to be carried out on the compounds.

Compliance with ethical standards

Acknowledgments

The authors appreciate the assistance of Dr. Marvis Erhunmwuse of Department of Chemistry, Frutarom (UK) Ltd, TS25 2DT, United Kingdom for running the spectra and the Department of Pharmaceutical Microbiology at the University of Benin who supplied the test organisms.

Disclosure of conflict of interest

The author declares no conflict of interest.

References

[1] Narayan UL, Nerkar AC and panda CS. (2006). Synthesis and screenings of 6, 6-methyl-3-(21raryl – thiazolin-4-one 1/2 – aryl – imidazolidin– 4 – one quinazolinon– 4 (3H) – ones for antibacterial and antifungal activities. Int. J. Chem Sci. 4(1), 93 – 100.

[2] Patlan SR, Reddy WK, Pattan JS, Venkataramanna NV, Prajapati PN and Hemashttar SM. (2005). Synthesis and microbiological evaluation of N1 – 3 (4 –(4 –chloro-phenyl thiasole – 2 –yl. Quiliasoline – 4 – (3H) – one. Lud J. Hetero. Chem. 15, 79 – 80.

[3] Marzoog SAL. Thebeiti, Maher F and EL – Zohry. (1998). Synthesis of some spirothiazolidinone and spirozetidinone derivatives incorporated with quinazolin. Ind. J. Chem. 37B, 804 – 809.

[4] Trilok Chandra, NehaCarg and Ashok Kumar. (2009). Synthesis of sulpha Drug Quinazolin – 4 –one Derivatives and their Evaluation for Anti – inflammatory Activity. W. J of Chem, 4 (2), 210 – 218.

[5] Peyman Salehi, MinooDebir, Mohammad Ali Zolfigol and Mostafabaghzadeh. (2005). A new approach to the facile synthesis mono and disubstituted quinazolines– 4 (3H) –ones under solvent free conditions. Tetrahedron Lett, 7051 – 7052.

[6] Kale AU, Kardile DP, Kalyane NV, Patel MR and Patel H. (2010). Synthesis and antimicrobial activity of some 2, 3 – disubstitutedquinazolines – 4(3H)-ones. Int. J. Pharma Applied Sci. (1).

[7] Deepth Kphli, Riazhashim, S, Sugar Viahal, manish Sharma and Ashutosh Humar Singh. (2009). Synthesis and antibacterial activity of quinazolines derivatives. Int. J. Pharm Sci.

[8] Mohamed MS, Kamel MM, Kassem E.M.M, Abotaleb N, Adb. El – Moez, SI and Ahmed MF. (2010). Novel 6, 8 – dibromo – 4 (3H) – quinazoline derivatives of anti – bacterial and anti – fungal activities. Eur. J. Med. Chem., 45, 3311 – 3319.

[9] Chavan BB, Bhalawane PP, Kolsure AK and Chabukswar AR. (2014). Synthesis and Evaluation of Some New 4, 6-Disubstituted Quinazoline Derivatives for Antimicrobial and Antifungal Activities. Asian Journal of Biomedical and Pharmaceutical Sciences, 04 (33), 43-46.

[10] Chandrika, PM., Yakaiah, T, Rao, AR., Narshaiah, B, Reddy NC, Sridhar, V and Rao JV. (2008). Synthesis of novel 4, 6 – disubstitutedquinazolines derivatives, their anti – inflammatory and anti – cancer activity (cytotoxic) against U937 Leukemia cell lines. Eur. J. Med Chem., 43, 46, 846 – 852.

[11] Giri, RS,Thaker, HM, Diordano T, Williams, J, Rogers D, Sudersanam V and Vasu KK. (2009). Design, synthesis and characterization of novel 2 – (2, 4 – substituted – thiazole– 5 –yl) – 3 – aryl – 3H –quinazoline–4 – one derivative as inhibitors of NF – KB and AP. 1 mediated transcription activation and as potential anti – inflammatory agents.Eur. J. Med Chem., 44, 2184 – 2189.

[12] Alafeefy AM., Kadi AA, Al –Deeb, OA, El – Tahir and KEH, Alijaber NA. (2010). Synthesis, analgesic and anti – inflammatory evaluation of some novel quinazoline derivatives. Eur. J. Med. Chem, 45, 4947–4952.
[13] Park HJ, Kim YS, Kim JS, Lee EK, YY, Hwuang HJ, Sih ME, Ryu CK and Lee SK. (2004). 6 - Arylamino – 7 – Chloro-quinazoline – 5, 8 – diones as novel cytotoxic and DNA topoisomerase inhibitory agents. Bioorg. Med. Chem. Lett., 14, 3385 – 3388.

[14] Jin Y, Zhou ZY, Tian W, Yu Q and Long, YQ. (2006) 4 – Alkoxyl substitution enhancing the anti – mitotic effect of 5 – (31, 41, 51 – substituted) aniline – 4 – hydroxyl – 8 – nitro – quinazoline as a novel class of anti – microtubule agents. Bioorg. Med. Chem. Lett., 16, 5864 – 5869.

[15] Kundu SK, Mahindaratne MPD, Quintero MV, Bao A and Negrote GR. (2008). One – pot reductive cydizationof antitumor quinazoline precursors. ARKIVOC, ii, 33 – 42.

[16] El – Azab AS, Al – Omar MA, Abdel – Aziz AAM, Abdel – Aziz NI, El – Sayed MAA, Sayed – Ahmed MM and Abdel – Hamid SG. (2010). Design synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents. Molecular docking study. Eur. J. Med. Chem., 45, 4188 – 4198.

[17] Alagarsamy V, Murugesu S, Dhanabal K. (2007). Anti – HIV antibacterial and antifungal activities of some novel 2 – methyl – 3 – (substituted methylamine) – (3H) + - quinazoline – 4 – ones. Indian J. Pharm. Sci., 69, 304 – 307.

[18] Jessy EM, Thirugnana A, Alex J. (2007). Synthesis and biological evaluation of some novel quinazolines. Indian J. Pharm. Sci., 69, 476 – 478.

[19] Patel NB and Barat GG. (2010). In vitro microbial studies of new pyrazolyquinazoline– (3H) ones. J, Saudi Chem. Sc, 14, 157-164.

[20] Georgey H, Abdel-Gawad N and abbas S. (2008) Synthesis and anticonvulsant activity of some quinazolin-4 (3H)-one derivatives. Molecules, 13, 2557 – 2569.

[21] Kashaw SK, Kashaw V, Mishra P, Jain NK and Stables JP. (2009). Synthesis, anticonvulsant and CNS depressant activity of some new bioactive 1- (4 – substituted-phenyl)-3-(4-oxo-2-phenyl/ethyl-4H-quinazolin-3-yl) – urea. Eur.J.Med.Chem, 44, 4335 – 4343.

[22] Alagarsamy V, Rajasolomon V, Meena R and Kona VR. (2005). Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2- Butyl-3-substituted quinazolin-4-(3H)-ones. Biol Pharm Bull, 28, 1091-1094.

[23] Shailaja KM, Dipukakoty, Shivakumar B, Jayachandran E, Sreenivasa and GM, Sriranga T. (2013).Synthesis and Characterization of Novel Heterocyclic compounds. Research and Reviews: Journal of Chemistry., ISSN: 23 19-9849.

[24] Okeke MI, Iroegbu CU, Eze E.N, Okoli AS and Esimeone CO. (2001). Evaluation of extracts of the root of Landolphia overriense for antibacterial activity. J. Ethnopharmacol 78, 119-127.

[25] Mackie R and McCartney. (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 OP. (2019). Synthesis and antibacterial activity of 3-amino-6-iodo-2-methyl quinazolin 4-(3H)-one and 6-iodo-2-methyl-4H-benzo [D] [1, 3] oxazin-4-one. World Journal of Advanced Research and Reviews, 2(3), 14-20.